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REACTION OF CUTANEOUS RECEPTORS TO LOCAL, REFLEX, AND GENERAL THERMAL INFLUENCES

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(Presented by Active Member AMN SSSR V. N. Chernigovskii)
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The significance of the thermoreceptive apparatus of the skin in the processes of heat regulation of the organism has not been studied sufficiently. And yet it is obvious that the first signals concerning changes in temperature, which lead to appropriate changes in the relationship between the processes of heat production and heat loss, emanate precisely from the cutaneous thermoreceptors.

METHODS

The method used for study of the reactions of the thermoreceptors utilized the examination of functional mobility. The research was carried out with the aid of cold and hot thermoesthesiometers, the diameter of the thermal probes being 1 mm. The method worked out earlier by Z. P. Belikova and O. D.Kolyutska, had been used for the finding or mapping out of (thermal) spots.

Previous use of this method, in the finding of thermosensitive points on the skin of the palmar surface of the forearm with the aid of an appropriate thermoesthesiometer, revealed 10-15 such cold and hot spots, which were marked with indelible dye or ink. This method of investigation is convenient for clarifying the reaction of the thermoreceptors to local or reflex action of thermal stimulation. In using the method for mapping the palmar surface of the forearm, the sensitive points were marked on a stamped grid, the intersecting lines of which formed 25 points. This was a convenient method for establishing the functional level of the thermoreceptors during differing ambient temperatures.

During investigation of cold receptors by means of successive touching of the cold thermoesthesiometer to each of the previously mapped out points, points were found in which the sensation of cold was detected, i.e. functional receptive elements for cold.

The research on warm receptors was carried out by this same method with the aid of a warm thermoesthesiometer. The method described allowed one to judge the number of functioning cold or warm receptive elements, i.e. the level of their mobilization at any particular instant of investigation.

Observations were carried out on five subjects.

After a 15-20 minute adaptation to the temperature of the experimental room, the background level of mobilization of the thermoreceptors was determined, after which observations were made on change of level of mobilization of the thermoreceptors of the skin during a particular thermal influence.

By means of the systematic method described, we investigated the reaction to cold and heat of the cutaneous receptive systems of man during local, reflex, and general action of a thermal stimulus.

RESULTS

Reaction of cutaneous thermoreceptors to local thermal stimulation. During investigation of the cutaneous receptive system for cold, a distinct reaction of the cold receptors was noted in response to a local thermal stimulus. This reaction was expressed as a significant reduction of the level of mobilization of the elements receptive for cold at the site of application of the cold or warm stimulation.

Local cooling was brought about either by an ice bath or a rubber bag filled with water (from 5 to 12°). For local warming the bag was filled with water at a temperature of 42° to 46°, or else a sun lamp was used. (In the latter case, tactile stimulation was avoided.)

In the upper part of Fig. 1, it can be seen that during a brief (2-5 minute) cooling of the right forearm, a rapid and significant demobilization of the receptive elements for cold took place at the site of application of the stimulus — the level of mobilization was significantly reduced in comparison with the control. In the investigation of the symmetrical portion of the skin of the forearm on the left side no such changes of level of mobilization of the receptive elements for cold were noted. On the right of the same figure, the reaction of the receptors for cold to local heating is seen. The reaction was also expressed as a reduction of the level of mobilization of the receptive elements for cold at the place where the warm stimulation was applied, and absence of a pronounced reaction on the other side.

In the investigation of the cutaneous receptive system for heat, the same reaction to local cooling or heating

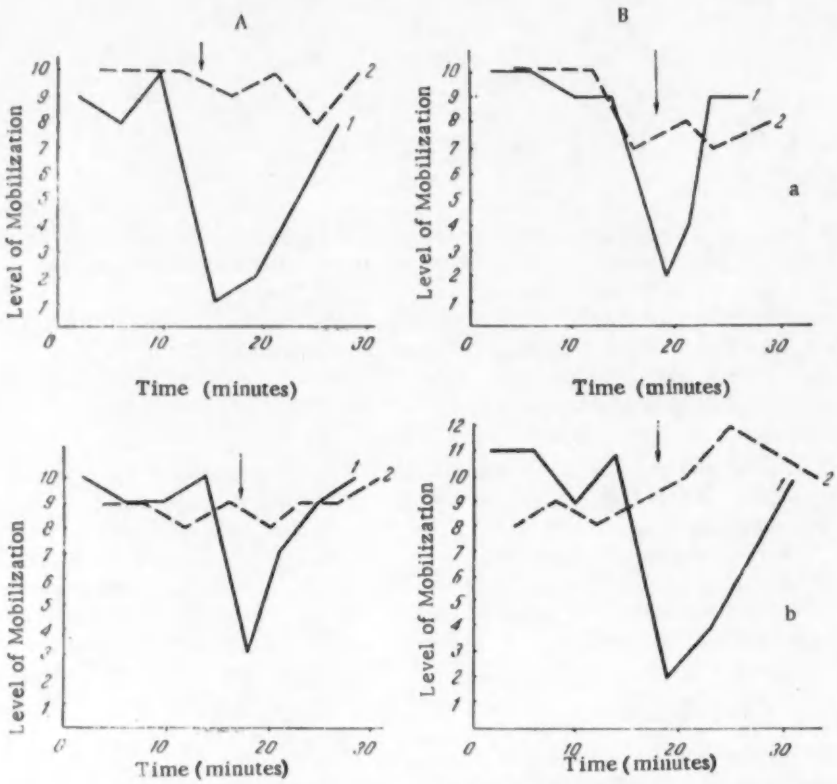

Fig. 1. Reaction of cutaneous thermoreceptors to local thermal stimulation. A) Local cooling; B) local heating. a) Cold reception; b) heat reception. 1) Right arm; 2) left arm.

was observed. The bottom part of Fig. 1 illustrates the reaction of the heat receptors to a local thermal stimulus. Both heat and cold led to a reduction of the level of mobilization of the receptive elements for heat at the place where thermal stimulation was given. Hardly any reaction was noted in the other arm. Thus, during a brief local cooling or warming, a rapid and significant demobilization of the receptors for both cold and heat is observed at the locus of application of a thermal stimulus. The reaction is local and not differentiated. No reflex variations of the temperature-sensing system of the skin is observed.

Reaction of the temperature receptors of the skin during reflex action of a thermal stimulus. With a longer lasting and more superficial thermal-stimulation (10-15 minutes) of the skin, a reflex effect of the thermoreceptive system was noted, and was expressed as a change of the tuning of the cutaneous thermal receptive apparatus which had not been exposed to the thermal influence. This reaction was noted during heating of the back with one meter infrared rays. The skin on the surface of the subject's forearm was not exposed to the warming action of the rays. In these cases, the reaction of the thermoreceptors was expressed as a lowering of the level of mobilization of cold and hot receptor elements of the skin of the arm to a warming, and a rise in their mobilization level during radiant cooling of the back. In Fig. 2, it can be seen that when a thermal stimulus brought about a reflex effect, a differentiated reaction of the thermo-

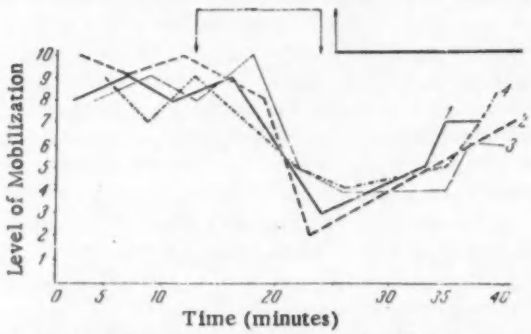

Fig. 2. Reflex reaction of the cold and heat receptors of the skin of the arm in response to heating and radiation cooling of the back. Heat reception of right (1) and left (2) arms. Cold reception of right (3) and left (4) arms.

↓ ↓ Heating:

↑ Cooling.

receptors to heating and cooling was observed: Reflex heating produced demobilization, while (reflex) cooling brought about a mobilization of the thermoreceptors.

The reflex lowering of the level of mobilization of the cutaneous thermoreceptors to infrared ray warming of the back was accompanied by a vigorous outpouring of sweat (the number of functioning sweat glands was increased), which is indicative of the increased heat loss.

The experimental data we have obtained can be interpreted as being due to the fact that during local, brief heating or cooling the temperature-regulating processes of the organism are not disrupted and therefore reactions are limited to local ones.

In those cases where heating of a significant square of skin on the surface of the body is achieved, some disturbance in the relationship between the thermoregulatory processes (heat production and heat loss) results. This leads to the reflex reduction of the level of mobilization of the cold-sensitive elements, which are located superficially and react to the change of the external temperature. The reduction of the level of mobilization of the heat-sensing elements observed during this situation evidently may be ascribed to the lowering of the temperature of the internal environment of the organism as a result of the increase in heat loss (increased sweat secretion).

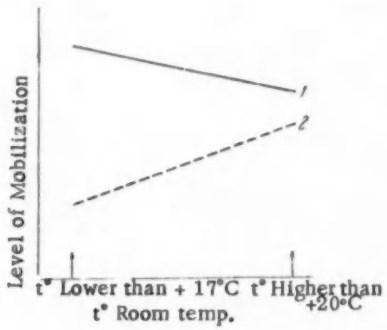

Fig. 3. Change of control functional level of the heat and cold receptive systems during different room temperatures. 1) Cold reception; 2) heat reception.

From the material we have presented, it is evident that during either local or reflex action of a thermal stimulus no reciprocity is manifested between the heat and cold-sensing receptive systems of the skin, the receptive system for cold being the main one which responds more quickly and actively to change in the external temperature. The system for heat reception is more passive and changes in it proceed in parallel with changes in cold reception.

P. G. Snyakin has suggested that the lack of reciprocal action could be explained by the fact that in our geographical region, with an average yearly temperature of 4-7°, the danger of excessive cooling is more of a threat to man than that of overheating. In connection with this, the cold-sensing system acquires a greater signalling significance than does the heat-sensing apparatus; and, second, Snyakin has suggested that the absence of reciprocity can be explained by anatomical and physiological peculiarities.

The receiving elements for cold are disposed more toward the surface, and their number significantly exceeds that of the heat receptors. Connected with this, there are also physiological features of one or the other system of thermoreceptors. The cold receptors found near the surface respond quickly to a change in the external temperature. The receiving apparatus for heat is more sensitive to changes in the internal temperature and react to a change in the heat balance of the organism. Therefore they react to changes in external temperature only when such changes lead to an alteration of the temperature of the internal environment of the organism.

Reaction of the thermoreceptors of the skin to the general action of thermal stimulation. Reciprocal action of the cold and heat receptive systems is observed during a generalized change of temperature in the surrounding environment. The level of mobilization of the heat receptors is significantly higher during elevated ambient temperature than when the surrounding temperature is low. In low ambient temperatures the level of mobilization of the cold receptors is increased in comparison with their level of mobilization in surroundings of high temperatures.

Figure 3 presents an average of the data from all experiments. Reciprocal dependence of the cold and heat receptors on changes in room temperature is obvious. It can be seen from the figure that when the room temperature is lower than 17°, the level of mobilization of the cold receptors is higher than in a room with higher temperature (as when the room temperature is higher than 20°). The reaction of the heat-sensitive receptive system to these same room temperatures changes in the opposite direction.

In our view, such a redistribution of the functional levels of the cold and heat receptor systems in large measure ensures maintenance of the constancy of temperature of the human body.

Thus, in order to preserve the homeothermy of the organism, these two thermoreceptive systems - for cold and heat - are set at a level corresponding to the particular functional state of the thermoregulatory centers. The reaction of the thermoreceptors ensures the adaptation of the organism to a change in temperature conditions. Functional mobility is one of the mechanisms contributing to the maintenance of a constant body temperature.

SUMMARY

In studying the reaction of thermoreceptors the author used the method of investigating the functional mobility. It was shown by observations that during local heating or cooling of short duration there occurs a rapid and considerable demobilization of the receptor elements responding to

both heat and cold at the site of application of the thermal stimuli. Evidently, in this case the thermoregulating processes of the organism are not disturbed and therefore the reaction is only local. With a prolonged action of T° stimulation extended over a larger surface of the skin - a reflex reaction of the thermoreceptor system is observed which is manifested in the changed adjustment of cutaneous thermoceptors, not subjected to the effect of thermal stimulation. In this instance a differentiated reaction to heating and cooling is being noted: heating provokes demobilization, while cooling - mobilization of the thermoreceptors. With this temperature changes of outside or inside air a reciprocal relationship is observed between the reaction of cold and heat receptor systems of the skin.

The data obtained demonstrate that the cold receptor system reacts more rapidly and adequately to changes of external temperature while the heat receptor system responds more readily to the temperature changes in the body.

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ELECTRICAL CHANGES IN THE DOG CORTEX DURING THE DEVELOPMENT OF DEFENSIVE CONDITIONED REFLEXES

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Recently, many electrophysiological studies of conditioned reflexes in man and in animals have been made.

Consistent potential changes in different parts of the brain have been found to occur during their formation. [1, 4 - 10, 12 - 18]. Attention has been frequently directed to changes in reactivity (in the magnitude of the electrical response) during the formation of conditioned reflexes. By using rhythmical stimulation, regular synchronized oscillations close to the stimulus rhythm or to multiples of it were observed [2, 6 - 10]. When the stimuli were presented irregularly, desynchronization usually occurred [5, 13]. However, insufficient attention has been paid to the distribution of electrical activity in the different parts of the cortex or to its changes during the development of conditioned reflexes.

It has been shown in rabbits [3, 9, 10] that during the formation of temporary connections in the sensory cortex there is a similarity in the electrical potentials corresponding to the associated stimuli. Subsequently, as the conditioned reflex becomes more accurately defined, the similarity becomes less marked.

In our experiments on dogs we associated two unrhythmical stimuli (light and passive lifting of the foot), and it was shown that first there was a similarity in the form of the electrical potentials of the cortical motor areas of the front and hind limbs, and that later the potentials in the visual cortex came to resemble those of the motor cortical centers.

The object of the present investigation was to study the relationships between the electrical activity in the different parts of the cortex in dogs in which a defensive conditioned reflex was established. We were particularly interested in those parts of the cortex which received no direct stimulus.

METHOD

The experiments were carried out on two dogs in a sound-proof room.

Both carried electrodes held by implants fixed to the skull bone by the method described by L. G. Trofimov and R. N. Lur'e [11].

The electrodes were arranged as follows: eight were implanted in the bone over the right hemisphere, four over the motor cortex (the third and fourth over the hindlimb area, and the fifth and sixth over the forelimb area, the eigth and ninth were placed over the auditory cortex, the seventh and tenth over the postcentral gyrus, and two electrodes were implanted in the bone over the left hemisphere over the visual cortex. The first was the indifferent electrode. However in most cases, bipolar recordings were made with an interelectrode distance of 3 mm.

The potentials were fed to amplifiers with symmetrical condenser resistance couplings, and recorded on a string oscillograph.

In dog No. 1 a set (stereotype) of defensive conditioned reflexes was developed as follows: Bell (+) 1,000 cps sound generator (+), 300 cps sound generator (-), 12 w electric lamp (+). In dog No. 2 the left foot was also used and separate conditioned defensive reflexes to sound and to light were elaborated. In all cases rhythmical stimuli were used. Reinforcement was given by electrical stimulation of the skin of the foot by an induction coil and at a voltage of 1 - 2 cm above threshold on the scale of the instrument, and the rhythm of the voltage applied was the same as that of the conditioned stimulus. The duration of the unconditioned (electrical) stimulus was one second.

The experiments were carried out every second day. In each experiment, four to five combined stimuli were applied.

RESULTS

In both dogs, before the elaboration of the conditioned reflexes, no resemblance was observed between the waves of the visual auditory, cutaneous, and motor cortex nor between the cortical areas corresponding to the front and hindlimbs (Fig. 1 a and b). These cortical areas responded very little to adequate stimuli. However, when a pattern of conditioned reflexes (stereotype) was developed in dog No. 1, even by the second experiment there was a marked response in the relevant cortical areas to the combined stimuli. At this time a change was first noticed in the relationships between the waves of the different cortical areas: there was some synchronization of these waves in the auditory cortex and hind limb

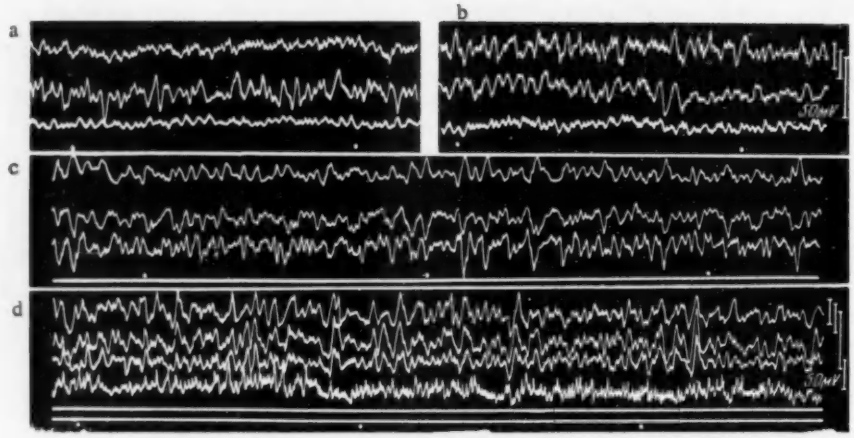

Fig. 1. Electroencephalogram of dog No. 1. Spontaneous activity. (Bipolar recording; time marker, 500 msec; calibration, 50μ v). a – Before developing the conditioned reflex. Curves, from above downwards; EEG of the hind limb cortical area; b – after developing the conditioned reflex. Curves, from above downwards: EEG of the cutaneous area, EEG of the forelimb area, EEG of the hind limb area; c – development of a conditioned reflex pattern (stereotype). Curves, from above downwards: EEG of cutaneous area, EEG of auditory area, EEG of hind limb area; d — development of stereotype. Curves, from above downwards: EEG of cutaneous area, EEG of auditory cortex, EEG of hind limb area, EEG of forelimb area.

areas (Fig. 1c lower trace), but from the electroencephalogram traces it appeared that the cutaneous area (Fig. 1c) was not yet influenced.

In the next experiment, in addition to the auditory cortex and hind-limb area, the general sensory area also became synchronized. However, the change was temporary and disappeared three to four minutes after the combined signals had been given. Not until the seventh experiment was there a definite synchronization which was maintained until the end of the experiment in both the auditory and hind-limb motor cortex (Figs. 1, 2).

The last change was that of the visual cortex. At the same time, the fore-limb area as before showed no synchronization (Fig. 1d and Fig. 2b). It should be noted that the involvement of the visual cortex is very short-lived, and is not maintained as well from one experiment to another as the activity in the hind limb, cutaneous, and auditory areas (Figs. 1d and 2b). Possibly the effect is due to the instability of the conditioned reflex to light as well as to the placement of the electrodes (they were implanted in the bone over the right visual cortex, while all the other electrodes were on the left side).

It was important that the conditioned stimulus abolished the synchronization. This occurred chiefly through reducing the response in the hind limb cutaneous area, and also through the appearance of rapid waves characteristic of the visual cortex (Fig. 2e).

When the stereotype became firmly established, the synchronization of the waves in the cutaneous and auditory cortex was maintained even during the action of the conditioned stimulus. When the regular combination of signals were no longer given, the time for the recovery of synchronization in the above mentioned cortical areas was from 20 to 90 sec. After recovery, synchronization was mantained until after the end of the experiment, i.e. for one – two hours.

At the same time as the disturbances in the relationship of the activity of the different parts of the cortex occurred there were also changes in their reactivity: At first the effect of the conditioned stimuli was to cause the appearance of electrical waves having a rhythm either equal to the stimulation frequency or to a multiple of it (Fig. 2c); subsequently, these same rhythms were found in the areas corresponding to the unconditioned stimulus (Fig. 2d), and finally occurred in the latter area only (Fig. 2e). At this time there was a fall in the reactivity in the forelimb cortical area.

In dog No. 2, defensive conditioned reflexes to both sound and light were elaborated.

In Fig. 2f it can be seen that there is a marked similarity in the waves of the visual cortex and motor areas (the electrodes were implanted only over the hind limb area). In response to the conditioned stimulus, in addition to the conditioned limb movement there were also synchronized potentials developed which had a frequency equal to that of the stimulus.

The synchronization of the waves of the different parts of the cortex occurred only after all preparations had been completed and the door of the room closed (see Fig. 2b).

The waves from these cortical areas recorded during the preparatory period show no similarity (Fig. 2a).

This result shows that the potential waves really do represent changes in the relationships of the different

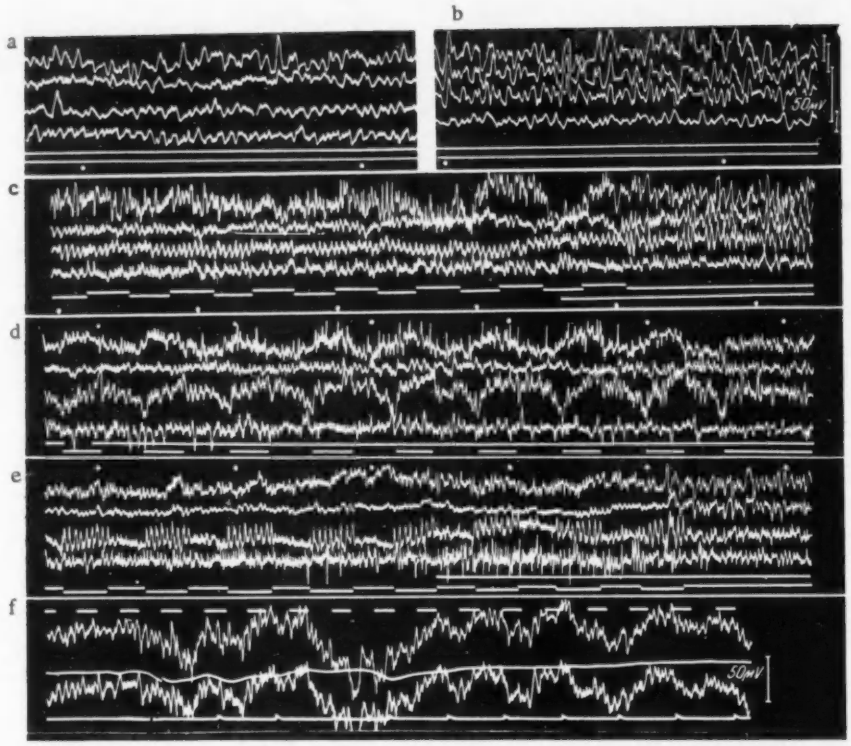

Fig. 2. Electroencephalograms of dog No. 1 (a – e) and dog No. 2 (f). Bipolar recording; time marker, 500 msec; calibration, 50μv). a Room open; b – two min after door was closed. The curves, from above downwards show EEGs of: auditory area, cutaneous representation, hind limb area, forelimb area, stimulus marker, record of limb movement; c – response to conditioned sound stimulus (indications as in Fig. 2 a,b); d – response to conditioned light stimulus. Curves, from above downwards show EEGs of: visual cortex, cutaneous representation, hind limb area, forelimb area, trace of limb movements, stimulus marker; e – response to sound conditioned stimulus. Curves, from above downwards show EEGs of: auditory cortex, cutaneous representation, hind limb area, visual cortex; record of limb movements, stimulus marker; c, d, and e are to be read from right to left; f – response to sound stimulation (monopolar recording). Curves, from above downwards: Time marker, 170 msec, EEG of hind limb area, trace of limb movements, EEG of auditory cortex, stimulus marker.

cortical areas and are of functional significance. Further evidence is that in a dog which in one experiment received the standard set of rhythmical stimulations, but at half the normal frequency, there was no disturbance of the electrical potentials. The reactivity of the cortial areas concerned and the magnitude of the conditioned reflexes were both greatly reduced. Subsequently, when the stimuli were once more given at the normal frequency, normal relationships were restored. Synchronization failure was also observed in another experiment when when the stereotype was altered by omitting the electrical reinforcement to the skin.

It must be emphasized that synchronization which developed through the formation of the conditioned reflexes was maintained subsequently; the electrical activity of the area representing the forelimbs never came into synchrony. This point was of special interest because the distance between the electrodes in the hind limb and forelimb areas was only 6 mm, whereas the distance between the hind limb area and the general sensory, auditory, and visual cortices had values from 16 to 45 mm. In our opinion, the results obtained agree with those which we obtained previously, and indicate that synchronization of the potentials is a sign of the formation of particular connections in the cortex during the development of coditioned reflexes.

SUMMARY

Electrodes were implanted into the skull over the different cortical sensory areas. It was found that in the development of defensive reflexes, a clear-cut synchronism in the potential waves occurred in the areas receiv-

ing the conditioned and unconditioned stimulations. This synchronism was very stable when a stereotype was elaborated. An important finding was that when training to the stimulus pattern resulted in movement of the hind limb, the electrical activity of the forelimb area never came into synchrony. These results indicate that connections developed in a dog's cerebral cortex during the elaboration of the conditioned reflex are selective even during the early stages of their formation.

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FORMATION OF A CENTRAL NERVOUS DOMINANT WHEN THE CONDITION OF THE NERVOUS CENTERS IS ALTERED BY DRUGS

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(Presented by Active Member of the AMN SSSR V. N. Chernigovskii)
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It has previously been reported from this laboratory [1, 2, 3] that as a central nervous dominant develops it passes through a number of phases related to changes in the ability of the center to summate stimuli. These results have been confirmed by other workers [9, 10].

We, and others, [4 - 8, 11, 12] have shown that as it develops further, the dominant passes into a condition of parabiosis.

The results obtained may be explained in terms of the changed condition of the centers resulting from the formation in them of a dominant.

To test this hypothesis, we have tried to find whether it is possible for a central nervous dominant to be formed while the nervous centers are influenced by drugs.

METHOD

Experiments were carried out on cats under sodium amytal and chloral hydrate anaesthesia. Sixty five to seventy five mg per kg of sodium amytal was injected subcutaneously. Two hundred to four hundred mg per kg of chloral hydrate were given per rectum or by mouth. A preparation was made of the semitendinosus muscles and peroneal nerves of both hind limbs and of the deep flexors of the digits and the ulnar nerves of both forelimbs. Myograms of the reflex contractions were recorded. The central cut ends of the nerves were stimulated electrically by an induction coil. The dominant was developed in the flexor center of the hind limb by prolonged subthreshold stimulation of the afferent fibers of the peroneal nerve.

The experiments were carried out in the following order:

1) The threshold stimulation of the peroneal and ulnar nerves required to cause reflex contraction of the semitendinosus and deep digital flexors of the fore- and hind-limbs was determined;

2) The contractions of these muscles for different stimulus strengths were recorded. In some experiments, while the peroneal nerve was being stimulated to cause contraction of the semitendinosous muscle, the peroneal nerve of the opposite side and both ulnar nerves were stimulated. Normally, stimulation of the opposite peroneal nerve inhibited the reflex muscular contraction, while stimulation of the ulnar nerve had no effect or else also inhibited this contraction. Next, the nerve was stimulated so as to form a dominant. At intervals of 15 - 25 min the stimulation was interrupted, and the previous test repeated. The formation of a dominant in the hind limb flexor center was indicated by increased contraction of the semitendinosus muscle caused by simultaneous "additional" stimulation of the opposite peroneal and the ulnar nerves.

RESULTS

The depth of anesthesia was determined from the corneal reflex, amplitude and frequency of respiration, and from the response to painful stimulation. The effect obtained depended upon the depth, and varied from one animal to another.

When the anesthesia was light and the reflexes were somewhat reduced, a dominant could be formed in the flexor centers of the spinal cord by prolonged afferent stimulation of the peroneal nerve. It could be developed simultaneously in both hind limb flexor centers. As can be seen from the myograms (Fig. 1 a) recorded 90 min after injecting chloral hydrate, "additional" stimulation of the opposite peroneal and the ulnar nerve inhibited reflex contraction of the semitendinosi.

The myogram of Fig. 1 b recorded 50 min after commencing subthreshold stimulation of the ipsilateral peroneal nerves showed that "additional" stimulation of the opposite peroneal and both ulnar nerves during reflex contraction of the semitendinosi causes an increased flexor reflex on both sides.

Further subthreshold stimulation of the nerve causes the dominant to become concentrated in the center receiving the subthreshold stimulation. This can be well seen from the next myogram (Fig. 1 c) recorded 90 min after the start of subthreshold stimulation; "additional"

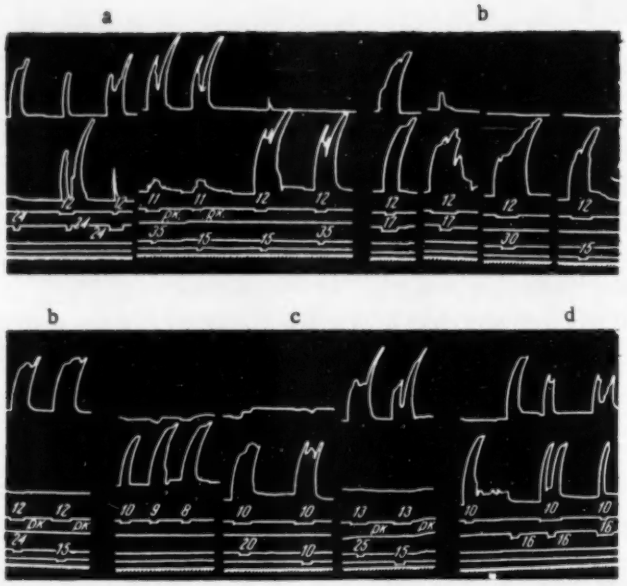

Fig. 1. Change in the semitendinosus flexor reflex caused by subthreshold stimulation of the ipsilateral peroneal nerve under light chloral hydrate anesthesia (formation of a dominant). a – Initial response, 90 min after injecting chloral hydrate; b – 50 min after applying subthreshold stimulation; c – 90 min after the onset of subthreshold stimulation; d – 150 min after subthreshold stimulation. Curves, from above downwards: Contraction of the contralateral semitendinosus muscle, of the ipsilateral semitendinosus, marker showing stimulation of the ipsilateral peroneal nerve, of the contralateral peroneal nerve, of the contralateral ulnar nerve, of the ipsilateral ulnar nerve, time marker (1 sec). The numbers over the stimulus marker indicate the distance between the induction coils in centimeters. [P. K.– stimulation of contralateral peroneal nerves.]

stimulation of the ulnar nerve results in an increased reflex contraction of the ipsilateral semitendinosus muscle, though the same stimulus causes no contraction in that of the opposite side. Fig. 1 c also shows that increasing the strength of the stimulus increases the strength of contraction, and that therefore the condition is not one of parabiosis.

When the nerve was stimulated for as long as 120 - 150 min or more, the dominant disappeared and there was once more reciprocal inhibition.

The myogram of Fig. 1 d was recorded 150 min after applying subthreshold stimuli, and it can be seen that there was no increase in the reflex contractions of either of the semitendinosi due to the "additional" peroneal stimulation, and that stimulation of the contralateral peroneal nerve produced a well-marked reciprocal inhibition.

In deeply anesthetized animals, reflexes were reduced and equalizing and paradoxical parabiotic stages developed in the spinal flexor centers. Under these conditions, parabiotic stages occurred in the flexor centers even before the application of subthreshold stimulation of the peroneal nerve.

Prolonged subthreshold stimulation of the peroneal nerves did not result in the formation of a dominant in the corresponding center but caused only a further reduction in reflex excitability, and a more profound parabiosis. Reciprocal inhibition was maintained until the end of the experiment.

In many experiments, before the subthreshold stimuli were applied during the anaesthesia, reflex excitability was reduced, though parabiotic stages either failed to develop or else developed as far as the equalizing stage only. Under these conditions subthreshold stimulation caused only an increased inhibition and a development of equalizing and paradoxical stages in the spinal flexor centers.

The myogram of Fig. 2a was recorded 90 min after injecting chloral hydrate, and before subthreshold stimulation was commenced; it can be seen that increasing stimu-

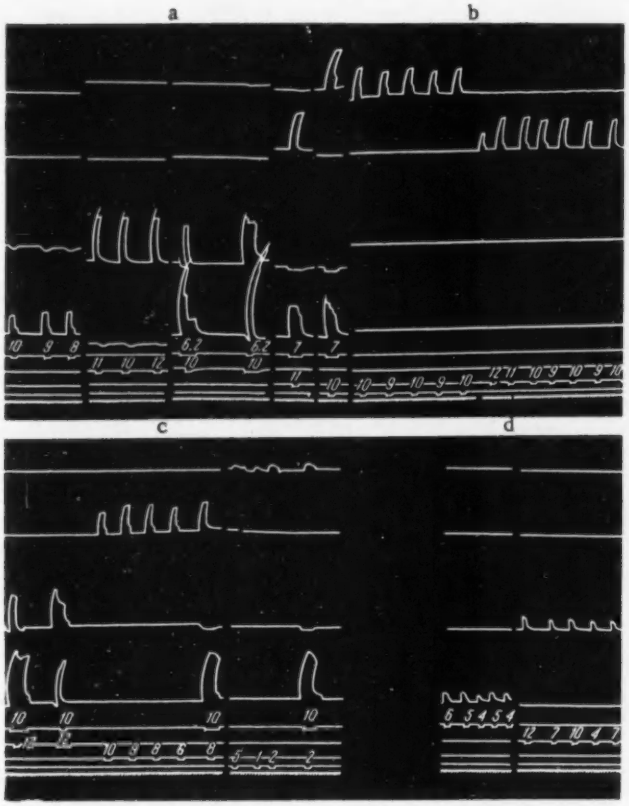

Fig. 2. Change in the reflex contraction of flexor muscles caused by subthreshold stimulation of the ipsilateral peroneal nerves in an animal under deep chloral hydrate anesthesia (no dominant formed). a – Initial response, 90 min after injecting chloral hydrate; b – 25 min, c – 60 min, and d – 180 min after commencing subthreshold stimulation. Curves, from above downwards: Contraction of the ipsilateral flexor digitorum profundus, contraction of the contralateral flexor digitorum profundus, of the contralateral semitendinosus, of the ipsilateral semitendinosus; marker of stimulus applied to ipsilateral peroneal nerve, to contralateral peroneal nerve, to contralateral ulnar nerve, to ipsilateral ulnar nerve; time marker (1 sec).

lation of the ipsilateral peroneal caused increased reflex contraction of the ipsilateral semitendinosus and that a stronger stimulus (9 cm on the induction coil scale) caused a stronger semitendinosus contraction than did a weaker stimulus (10 cm on the scale). Stimulating the contralateral peroneal nerve at a strength corresponding to 10, 11, and 12 cm distance between the coils caused a reflex contraction of the contralateral semitendinosus of almost the same strength. Here, as can be seen from Fig. 2a, the "additional" stimulation of the ipsilateral peroneal during contraction of the contralateral semitendinosus inhibited the flexor reflex. "Additional" stimulation of the ulnar nerve during reflex semitendinosus contraction had no effect on the amount of the contraction.

The next myogram (Fig. 2b) recorded 25 min after the onset of subthreshold stimulation shows the development of a paradoxical stage in the front limb flexor centers. Here stronger stimulation (9 cm) caused a weaker contraction than did the weaker stimulus (10 - 11 cm). Sixty minutes after the onset of the action of the subthreshold stimulus, reciprocal inhibition and parabiotic stages once more occurred (Fig. 2c).

Figure 2d was recorded 180 min after the onset of subthreshold stimulation, and it can be seen that the reflex contractions are now much weaker, and that paradoxical stages have occurred in the hind limb flexor centers. Strong stimulation of the ipsilateral peroneal nerve with a coil separation of 4 cm caused a smaller reflex contraction of the ipsilateral semitendinosus than

did stimulation when the separation is 5 - 6 cm. Strong stimulation with a coil separation of 4 - 7 cm applied to the contralateral peroneal nerves caused a contraction of the ipsilateral peroneal which was less than that which occurred with a weaker stimulation (10 - 12 cm separation). Under these conditions, reciprocal inhibition may be observed.

Similar observations were made on animals under sodium amytal anaesthesia.

It is important to note that during general anesthesia, the flexor centers of both fore- and hind limbs became involved in the parabiotic process as is shown by the fact that parabiotic stages are revealed by reflex excitability tests of these centers. The formation of a central nervous dominant is therefore possible under light anaesthesia, though it is considerably hindered by changes in the general excitability of the nervous centers. Under these conditions, the dominant is not stable and disappears with continued subthreshold stimulation. Under deeper anaesthesia, the excitability and liability of the spinal centers becomes greatly reduced, so that the conditions are not appropriate to the formation of dominant centers in the spinal cord. Subthreshold stimulation of a nerve now no longer induces the formation of a dominant, and leads only to an increased depth of parabiosis in the nervous centers.

When a certain depth of anesthesia has been reached, if the nervous centers are affected there is a marked reduction in their excitability and lability and it is no longer possible to establish dominant zones in the central nervous system, and previously established dominants are suppressed.

SUMMARY

Changes in nervous centers and the formation of a spinal cord dominant under the effect of chloral hydrate and sodium amytal were studied. The results showed that during subliminal stimulation of the peroneal nerve the spinal cord flexor centers could be stimulated.

When subthreshold stimulation was continued for about two hours, the dominant disappeared and was replaced by reciprocal inhibition. When anaesthesia was deep, both the reflexes and the depth of parabiosis in the flexor centers of the spinal cord were decreased. Subliminal stimulation applied to a nerve under these conditions did not induce a spinal cord dominant, but served only to cause an even greater reduction of the reflex excitation, and to intensify the parabiotic stages.

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INFLUENCE OF SMALL INTESTINE INTEROCEPTORS ON URINARY EXCRETION

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Many authors [1 - 7] have shown that a variety of reflex influences on urinary excretion may originate from receptors of the gastric mucosa.

The present work is a study of similar effects mediated by the small intestine.

METHOD

The experiments were carried out on three female dogs bearing ureteric fistulas established by I.P. Pavlov's method as well as Thiry-Vella intestinal fistulas. The latter were inserted 10 - 12 cm below the duodenum. The interoceptor was stimulated by perfusing an isolated loop of intestine with tap water at 36° and a rate of flow of 600 ml per 5 min. The water flowed evenly and silently from a graduated cylinder placed at a height of 1.5 m. As a control against possible absorption, the outflow was measured and found to be equal to the inflow.

The experiments were performed 16 hr after a meal. First a check was made to see whether the animals were thirsty; as a rule they refused water showing that they were not.

Urine was collected at 10 min intervals and the solid residue measured by a refraction method. In many experiments glomerular filtration and tubular reabsorption was measured by determining the endogenous creatinine.

During the first hour, no response from the animal occurred. The amount of urine secreted during this period was taken as the "spontaneous" excretion. At the end of the second hour the isolated intestinal loop was perfused, and measurements of urinary excretion continued.

In the first set of experiments, the effect of perfusion of the isolated loop on "spontaneous" excretion was determined. The animals were maintained on a constant food and water ration. The initial diuresis varied from 2.1 to 1.5 ml per kg per hr.

In the second set of experiments the effect on urinary secretion of perfusing the intestinal loop was observed while the animal was stimulated by food; the dogs were given 30 g of meat powder 10 min before the perfusion started.

In the third set of experiments, urinary excretion was measured during intestinal perfusion when there was a low initial diuresis. In Mushka and Nerka, the diuresis varied between 0.96 and 0.60 ml, and in Mirta between 0.60 and 0.42 ml per kg weight per hr. The low level was caused by restricting water intake 24 hr before the experiment.

In the fourth set of experiments, urinary secretion was measured after perfusing the intestinal loop with a 2% novocain solution.

RESULTS

The results of the first set of experiments showed that perfusion of an intestinal loop with tap water for 5 min causes a regular increase in diuresis which lasts, on average, for 30 – 50 min (Fig. 1). During the first 10 min after perfusion, diuresis was either increased or showed no change, and might even be reduced. After 20 min, as a rule, there was an increase in the diuresis and the maximum rate occurred 20 – 30 min after perfusion.

After the diuresis had increased, it usually returned to a level below the original value. The amount of solid substances was reduced with increased diuresis. There was also some reduction in the absolute amount of the solid residue after perfusion.

By measuring filtration rate and tubular reabsorption, it was shown that the increased diuresis occurs chiefly as a result of reduction in the latter.

In the second group of experiments, in which the animals were stimulated by feeding, perfusion had no stimulating effect on the diuresis (see Table). It must be noted that the act of eating dry substances itself reduces diuresis.

The results obtained in the second group of experiments show that under identical conditions the response may be very different according to the state of the animals.

In the third group, the influence of the intestine on diuresis was investigated when the initial urinary excretion rate was low. It was found that perfusion then caused only a very small increase in diuresis.

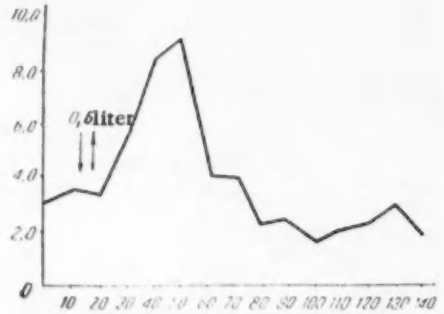

Fig. 1. Change in the spontaneous urinary excretion after perfusing an isolated loop of small intestine in the dog Nerka. Ordinate – diuresis in ml; abscissa – time in min. The arrows indicate the onset (↓) and end (↑) of perfusion; the figures over the arrows indicate the amount of water used.

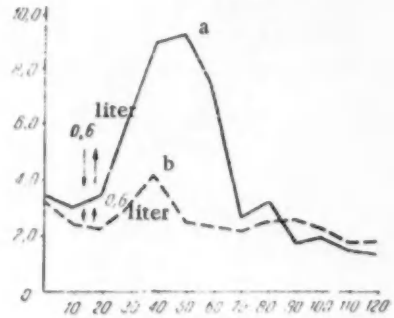

Fig. 2. Change in the spontaneous urinary excretion after perfusing an isolated loop of small intestine with (a) water and (b) a 2% novocain solution; dog Muska. Indications as in Fig. 1.

In some experiments no increase was found.

In the fourth set of experiments, it was shown that the receptors of the mucosa were concerned in the observed perfusion effects. Here the perfusion was carried out not with water but with a 2% novocain solution.

Figure 2 shows the results of an experiment in which an isolated loop of intestine was irrigated (a) with tap water, and (b) with a novocain solution.

As can be seen from the results, irrigation with novocain causes an increase in diuresis which is very small in comparison with the control experiment. There is no doubt that the reduced stimulant effect in this case is due to partial elimination of the mucosal receptors. The increased diuresis in the first group of experiments must be interpreted as a reflex reaction on the kidneys originating in the mucosal receptors of the irrigated section.

The second and third sets of experiments indicate that alteration in the functional condition of the body caused by reducing the water supply on the day of the experiment and by food stimulation inhibits the reflex stimulation of diuresis by the upper portion of the small intestine.

TABLE 1. Change in the Diuresis (in ml) in Dogs Measured at 10 min Intervals During Perfusion of the Mucosa of an Isolated Loop of Small Intestine With Water During Excitation by Feeding

Date of experiment	Name of animal	Before perfusion						After feeding		After perfusion							
		10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
1957																	
11/12	Nerka	3,9	3,7	4,1	3,6	3,1	3,0	1,5	0,9	1,9	2,1	1,7	1,6	2,0	1,8	1,4	1,1	1,3	1,6
11/13	"	5,0	3,9	3,5	3,8	3,3	2,9	0,7	1,6	1,3	1,9	1,7	2,4	1,8	2,0	1,9	1,9	1,6	1,2
11/19	"	4,4	4,2	5,0	3,9	3,7	3,0	1,0	1,3	1,7	1,6	2,1	2,5	2,0	1,8	1,6	1,4	—	—
11/12	Mushka	3,3	3,4	3,0	2,7	2,9	2,6	0,9	0,8	1,1	1,0	1,6	1,4	1,5	1,2	1,3	1,1	0,9	1,1
11/13	"	4,0	4,3	3,6	3,4	3,0	2,5	1,3	1,0	0,9	1,6	1,9	2,0	1,7	1,2	1,1	0,9	0,8	1,0
11/15	"	4,1	3,5	3,2	3,3	3,0	2,7	1,6	0,7	0,9	1,3	1,5	1,3	1,7	1,6	1,4	1,2	1,1	1,2
11/19	"	3,4	3,2	2,7	3,0	2,9	2,4	1,2	0,9	1,2	1,1	1,6	1,4	1,3	1,7	1,6	1,0	0,9	1,2
11/14	Mirta	2,0	2,4	2,1	1,9	2,0	1,7	0,6	0,9	1,0	0,9	1,3	1,2	0,9	1,0	1,5	1,4	1,0	0,8
11/15	"	2,6	2,7	2,4	2,1	2,0	1,9	1,0	0,8	0,9	1,4	1,6	1,2	1,1	1,0	1,1	1,0	0,9	1,1
11/20	"	3,0	2,6	2,5	2,3	2,4	2,1	1,3	0,9	0,8	1,3	1,1	1,6	1,5	1,7	1,0	1,2	0,9	1,0

SUMMARY

The effect of water perfusion of a Thiry-Vella loop of small intestine was studied in three dogs in chronic preparations. As a rule, when urinary excretion was at the rapid rate of 2.1 - 1.5 ml per kg body weight per hr, it was increased by perfusion. The reaction was reflex in nature and was mediated from the mucous membrane of the perfused intestinal loop. This finding was confirmed by perfusing with a 2% novocain solution, when no rise in diuresis occurred.

The reflex from the mucous membrane of the small intestine on the kidneys depends largely on the initial functional condition of the drinking and food centers. Water perfusion of the isolated portion of the small intestine had no stimulating effect on diuresis either under conditions of food excitation or when the water content of the body was reduced.

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THE PART PLAYED BY VASCULAR SPASM IN DISTURBING THE ANTICOAGULATING SYSTEM OF THE BLOOD

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Thrombosis and embolisms constitute one of the most important unsolved clinical problems. In spite of the efforts of clinicians, biochemists, pathologists, and physiologists, the cause of the formation of thrombi remains unknown. Suggestions have been made as to the possible significance of arrest of the blood flow in the greater or lesser circulation [5, 9], or of an increase in protein thrombogenic factors [7, 11, 13, 14], or of changes in the vascular wall [1, 2, 8]. However, all these ideas originate from clinical observations or from the findings of morbid anatomy and are not based on experiment.

The proposal put forward by B. A. Kudryashov and his coworkers that in man and animals there is a special physiological anticoagulating system appears to hold great promise. It was found that intravenous injection of even moderate doses of thrombin does not produce the normal reaction of forming thrombi, but as B. A. Kudryashov and P. D. Ulitinaya [4] have shown, on the contrary, the blood loses its ability to coagulate. The explanation that has been put forward is that the presence of excessive amounts of thrombin stimulates a reflex mechanism which has the effect of liberating into the blood stream humoral factors which eliminate the coagulating mechanism from the blood. It is not known in what way this system is brought into play nor on what substances its anticoagulating action depends. It is observed only that there is a fall in thromboplastic activity and fibrinogen content, and an increase in the amount of heparin-like substances. Kwann and his coworkers produced experimental thrombi in the limb vessels and observed that normally they were lysed in 9 – 32 hr; they ascribed this change to the secretion of cholinergic substances.

We have studied the anticoagulating system in several diseases to find what is the anticoagulating effect in vascular spasm. Clinically, it is thought that increased blood coagulation is an important factor in thrombus formation, and many consider it to be the principal cause of thromboembolic complication. If it is assumed that there is an anticoagulating system, then increasing coagulating power will bring about an opposite response from the organism and can therefore not by itself lead to the formation of thrombi. The object of the present investigation is to determine the nature of the accessory factors which, although not playing the main part, may, at any rate, be of critical importance in the formation of intravascular thrombi.

METHOD

The experiments were carried out on 29 Chinchilla rabbits weighing from 1.7 to 2.3 kg. Standard thrombin produced by the N. F. Gamalya Institute of Microbiology and Epidemiology was used. Before the experiment, 1.8 cc of dry thrombin from an ampoule was diluted in 10 g of physiological saline. The activity of the thrombin solution was measured by the time taken for 0.1 ml of the solution to coagulate 0.1 ml of oxalated blood at 37 deg. The activity was found to vary very little, and the coagulating time ranged from 10 to 12 sec.

First of all, the maximum dose of thrombin whose intravenous injection caused no thrombi to develop was determined; when injected, the blood actually lost its clotting power. The determination was made by injecting different amounts of thrombin solution into an ear vein. As can be seen from the table below, 4 ml of thrombin solution was the maximum dose which did not produce thrombi.

Number of Animals	Amount of thrombin solution (in g)	Effect of Injection
3	8	All animals died
4	6	Three rabbits died, one survived
4	4	All animals survived

The blood of the surviving animals failed to coagulate in 60 — 80 min. This confirms the existence in rabbits of the physiological anticoagulating system discovered by B. A. Kudryashov and P. D. Ulitina in rats.

We have tried to find whether this protective reaction is maintained in vascular spasm, which appears to be the principal factor in many pathological con-

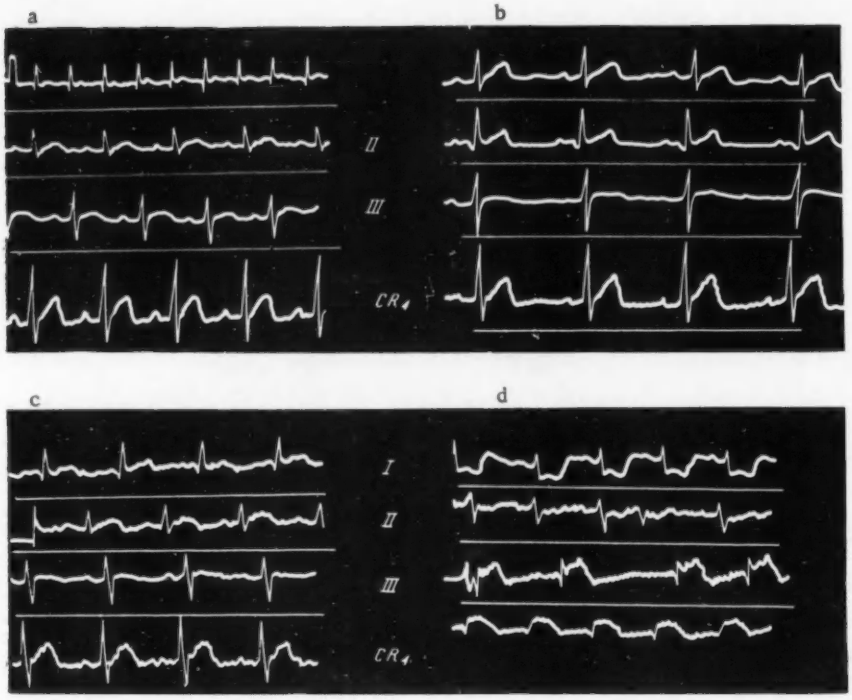

Fig. 1. Electrocardiograms after intravenous injection of (c) thrombin, (b) pituitrin, (d) simultaneous injection of thrombin and pituitrin; (a — normal). I, II, III — the three leads.

ditions. Pituitrin, whose vasoconstrictor effect has been known since the time of Krogh's classical work, was used to raise the blood pressure. Because pituitrin causes a pressor response in the coronary arteries [2, 15, 16], we decided to record the electrocardiogram so as to be able to detect the development of experimentally induced coronary thrombis and the resulting ischemia and myocordial infarction. Control observations on the ten rabbits showed that 0.5 cc of pituitrin injected intravenously caused no ischemia or cardiac infarction and that death did not ensue, so that there was no possibility of thrombi being formed in the vessels (Fig. 1). Here it can be seen that intravenous injection of 4 ml of a thrombin solution had little effect on the e.c.g.

Thus, neither the increased amount of thrombin nor pituitrin when given separately caused any thrombus formation. Immediately after the control experiments, the following procedure was carried out. A simultaneous injection of 4 ml of a thrombin solution and 0.4 cc of pituitrin was given into an ear vein. Four animals died immediately; post mortem examination revealed extensive thrombosis in different parts of the vascular system. In the remaining 17 animals, changes in the electrocardiogram typical of acute coronary damage and myocardial infarction were revealed (see Fig. 1). The e.c.g. changes indicated extensive myocardial damage associated with acute coronary thrombosis. Of the 21 animals used, seven died within 1 hr, six in the first 2 hr, three on the first day, and one on the second day. Coronary thromboses were found in all the animals post mortem and were confirmed microscopically. Figure 2 shows the thrombi formed directly at the origin of the aorta.

Histological investigation revealed typical acute coronary insufficiency and the onset of myocardial infarction. In seven cases only thrombosis of the coronary vessels was found, in eight cases there was also a thrombosis of the pulmonary artery, and in two animals thromboses were also found in the vessels of the abdominal cavity. It was found that small doses of heparin given after the development of the thrombus reduced subsequent intravascular thrombus formation. On this account, it was possible to enable four of the rabbits with signs of acute myocardial infarct to survive, though only for a short time, and to reduce the extent of the ischemic area. This method enables the damage to the myocardium to be limited.

The results obtained leave no doubt that vascular spasm prevents the development of the protective anticoagulating reaction, and allows intravascular thrombus formation to take place when the blood-thrombin content is increased.

The formation of thrombi in the coronary vessels shows that when the vessels are in spasm and clotting

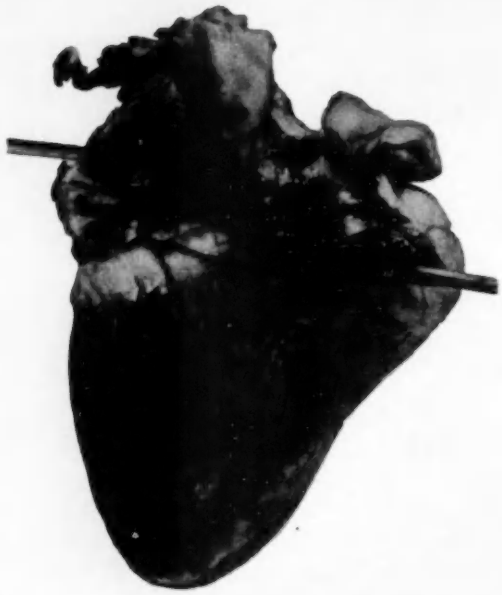

Fig. 2. Thrombosis of the coronary artery.

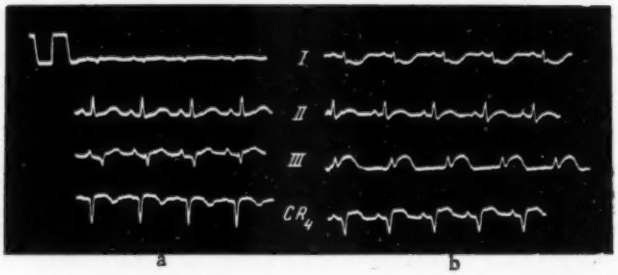

Fig. 3. E.c.g. of rabbit with 30 day myocardial infarct (a) before and (b) after intravenous thrombin injection.

power is raised, thrombi may develop independently of any changes in the walls. This conclusion is confirmed by other investigators [10] who found no changes in the vessel walls in a number of cases of myocardial infarction.

It has been shown [6] that in myocardial infarction, the coronary vessels are in a spastic condition which alters their response. In view of the high incidence of thromboembolic complications and repeated coronary thromboses, we investigated the anticoagulating reaction in myocardial infarction. We were especially interested in the period after the 30th day, when the e.c.g. returned to normal. The observations were made on nine rabbits in which myocardial infarction was induced by ligaturing the coronary artery.

As we had shown previously, injecting healthy rabbits with 4 ml thrombin solution causes no thrombus formation. In the experimental animals, in four cases the same thrombin injection produced electrocardiographic changes typical of repeated myocardial infarction. In five cases, 2 g of thrombin solution were sufficient to cause intravascular thrombus formation and coronary thrombosis (Fig. 3).

All nine rabbits died in the first 24 hr. Four received 2 g of thrombin solution, and in them the thrombus was found only in the coronary vessel; in the others they were found elsewhere in the vascular system as well; in one case, three hours after the development of myocardial infarction and marked physical signs, there was a split in the heart at the boundary of the scar tissue developed after the first infarction and the region affected by the coronary disturbance.

The results show that intravascular thrombus formation occurs more regularly after a myocardial infarction has developed. In such cases a second infarction and thromboembolic complications may be induced by increasing the clotting power of the blood. This fact confirms the clinical observations on the necessity for prolonged anticoagulation therapy extending into the post-infarction period.

Our conclusions are as follows: The development of vascular spasm interferes with the anticoagulating system and brings about intravascular thrombus formation; if spasm of the coronary arteries occurs when the blood-clotting power of the blood has been increased, myocardial infarction may develop without there being any changes in the walls of the coronary arteries; after the coronary infarction has developed, the altered condition of the anticoagulating system has the effect that increasing the coagulating power of the blood may now bring about thromboembolic complications and repeated coronary thrombosis.

SUMMARY

The effect of the vascular spasm on the function of the anticoagulating system of the blood was studied in experiments on rabbits. Intravenous injection of thrombin solution (4 g) into healthy animals did not provoke thrombosis; on the contrary, the blood lost its ability to coagulate. However the same dose of thrombin together with 0.5 ml of pituitrin resulted in the rapid formation of intravascular thrombi causing coronary thrombosis and myocardial infarction. A study was made of the extent of the possible action of the anticoagulation system in myocardial infarction which was induced by ligation of the coronary arteries. The administration of 4 g of thrombin induced a repeated infarction, and thrombosis in various parts of the vascular system. It was concluded that: a) The anticoagulation system of the blood becomes disturbed during vascular spasm, so that there is an increased tendency to intravascular thrombus formation; b) myocardial infarction may occur without changes in the walls of the coronary vessels only when there is spasm which causes an increase in the tendency of the blood to coagulate. Myocardial infarction itself will alter the anticoagulatory system of the blood, so that intravascular thrombus formation may be induced by small doses of thrombin administered alone.

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THROMBOKINASE ACTIVITY OF TISSUES IN VARIOUS ENDOCRINE DISORDERS AND IN TUMOR GROWTHS

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There are several references [4, 5, 6, 8] to a possible association between tumor development and endocrine disorders; it seemed worthwhile, therefore, to study the effect of hormonal disturbances on the properties of tissues which are related to tumor growth.

One such property is evidently thrombokinase activity, because there is evidence that thrombokinase has a stimulating effect on the growth and division of cells in tissue culture [10, 11], as well as on the thromboplastic properties of the experimental carcinoma V2 in the rabbit.

We have not found any reference to the work on the relationship of thrombokinase activity and the condition of the endocrine system, though there are isolated references [12, 13] to the fact that hormones may affect several components of the blood coagulatory system. There is no information about the thrombokinase activity of the tissues of many experimentally induced tumors or of the other tissues of the animal affected.

We therefore investigated thrombokinase activity in the tissues of rats with experimentally induced endocrine disorders and with sarcoma strains M-1, sarcoma 45, and with Biskind's ovarian tumors.

METHOD

Experiments were carried out on 68 white rats of an impure strain: Of these, 50 males weighed 250 - 300 g and the 18 females 200 - 250 g. The endocrine disturbance was induced by thyroidectomy, adrenalectomy, or castration. We also used castrated animals with ovarian tumors, as obtained in special researches on the transplantation of an ovary into the spleen using Biskind's method.

The sarcomas were induced by introducing a suspension of M-1 and 45 sarcoma tissue.

The animals were killed by decapitation after 1, 2, and 4 weeks, while those operated by Biskind's method and the control castrates were killed after 6 months. In a separate experimental group, blood was first taken from the jugular vein [1] to determine the thrombokinase (thromboplastic) activity of the blood using B. A. Kudryashov and P. D. Ulitina's method [3].

The thrombokinase activity of the tissue extracts was measured by Quick's method [14]. The relative concentration of thrombokinase in the extracts was determined by the minimum dilution of the extract necessary to destroy its activity [7].

The thrombokinase was tested on rabbit plasma containing a large amount of prothrombin [15] as well as of the thrombotropin [2] necessary for the activation of the inactive form of thrombokinase (prothrombokinase). The rabbit blood was taken from the heart by a syringe containing 0.1M oxalate solution in 1 ml per 10 ml of blood.

RESULTS

All the animals were divided into groups: 10 controls, 11 castrates six female castrates with one ovary transplanted into the spleen, 10 thyroidectomized, 10 from which two-thirds of the adrenal was removed, 10 totally adrenalectomized, six with sarcoma M-1, and five with sarcoma 45.

Study of the tissue extracts of the intact controls showed that each white rat tissue had a particular value of thrombokinase activity. The greatest was that of the lung (Fig. 1a, I) and the next in order were the tissues of the testes, brain, kidneys, and spleen (Fig. 1a – d, I). The liver had least. (Fig. 1f, I). Blood was intermediate in this respect between the kidneys and spleen.

Thrombokinase activity in corresponding tissues from different individuals varied by ± 2.5 sec for tissues with high and by ± 6 sec for those with low thrombokinase activity; blood showed variations from one individual to another of ± 10 sec.

Experiments on rats from whom different endocrine glands had been extirpated showed that the change in thrombokinase activity of their tissues was greater than the individual variations in the control group.

Castration caused an increase in activity chiefly in the lungs (Fig. 1a, K) and blood (Fig. 1g, K). Tissue extracts from castrated females with one ovary transplanted into the spleen did not show the increased thrombokinase activity of the other castrates (Fig. 1, B).

Thrombokinase Activity of Human Tumor Tissue

Tissue	No. of cases of the condition	Clotting time of control plasma	Thrombokinase activity (in sec)	
			tumor extracts	surrounding healthy tissue
Metastasis of omentum (cancer of the sigmoid colon)	1.109	67	16	–
Metastasis of omentum (cancer of the cecum)	1.069	100	25	–
Cancer of the stomach	1.097	90	18	–
Cancer of the breast	1,253	80	21	–
"	1.338	80	43	50
"	1.244	80	13	27
"	899	100	42	62
"	1.272	80	48	59
"	1.283	70	46	58
"	1 450	80	50	62
"	13.183	70	40	43
"	1.702	70	42	48
"	1.406	72	45	53
"	1.755	65	41	–
"	1.562	90	46	62

Thyroidectomy caused chiefly an increase in the thrombokinase activity of the blood (Fig. 1g, T), kidneys, and spleen (Fig. 1, T, g). There was little change in that of the lungs or brain in most of the thyroidectomized animals, and in the testes it was actually reduced (Fig. 1b, T).

The remarked changes in the activity of all tissues, and particularly that of the lungs, kidneys and brain, occurred in the adrenalectomized group (Fig. 1a, A, and d, c, A).

The experiments in which the extracts were diluted showed that the increased thrombokinase activity represented a disproportionate increase in the concentration of thrombokinase in the extracts (Fig. 2). For instance, whereas an extract of pulmonary tissue from the control (intact) group of animals reduced the clotting time of control plasma to 21 sec, an extract of castrate pulmonary tissue to 13 sec, and pulmonary tissue from an adrenalectomized animal to 10 sec (see Fig. 1), the correspondingly diluted extracts which failed to speed clotting were: for pulmonary tissue of the control (intact) animals – $10^{2.41}$, i.e. a 256-fold dilution, for castrate lung tissue – $10^{3.91}$, (8192 times dilution), and for pulmonary tissue of the adrenalectomized group – $10^{4.52}$, (32708 times dilution) (Fig. 2a, I, K, A).

The graph of Fig. 2 showing the relative concentration of thrombokinase in the extracts, where the concentration is shown as the logarithm of the dilution, illustrates the close relationship between the thrombokinase (more accurately prothrombokinase) content in the tissues and endocrine imbalance. Apparently individual variation in thrombokinase activity which may occur in control animals is also associated with endocrine balance.

In animals bearing tumors, the changes in thrombokinase activity were found to be much smaller than in those where there had been endocrine disorders (see Fig. 1, M-1, S-45). Only the blood of the sarcoma group, and particularly those with sarcoma M-1 showed a marked increase in thrombokinase activity (see Fig. 1, g, M-1, S-45).

Extracts of the tumors themselves of both kinds showed very high thrombokinase activity (see Fig. 1, 3, M-1). Extracts of sarcomatous tissue did not lose their activity until the dilution was $10^{4.21}$ and $10^{4.52}$ (see Fig. 2, 3, M-1, S-45), in other words the thrombokinase concentration in sarcomatous tissue approached the highest level i.e. that of pulmonary tissue in the adrenalectomized group.

Because of the results obtained on the thrombokinetic properties of the experimental sarcomas, we thought it

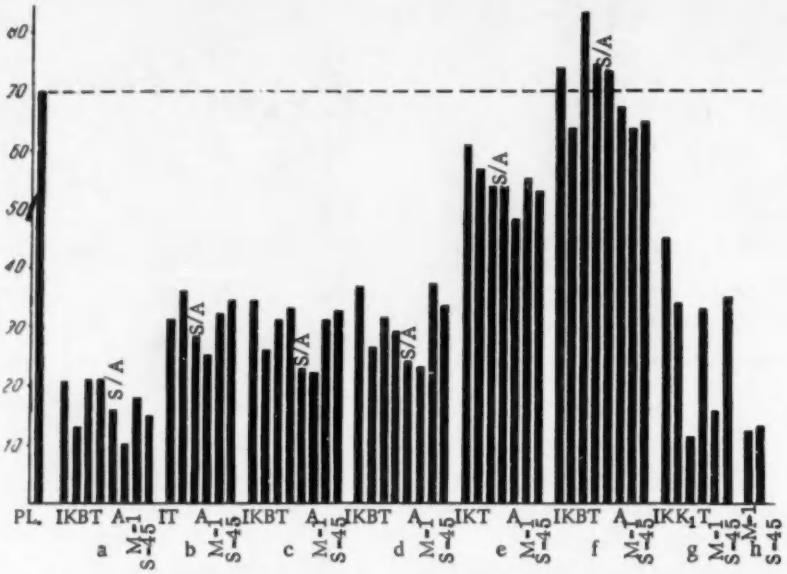

Fig. 1. Changes in the thrombokinase activity of different tissues as shown by the clotting time (in sec) of the control plasma (average values). a – Tissues of lung; b – of testes; c – of brain; d – of kidneys; e – of spleen; f – of liver; g – of blood; h – of tumor. Pl – control oxalate rabbit plasma; clotting time after recalcifying shown by dotted line, I – control (intact) animals, K – castrated males, K_1 – castrated females, B – females operated by Biskind's method, T – thyroidectomized animals, S/A – subtotally adrenalectomized animals; A – completely adrenalectomized animals, M-1 – animals with 14-1 sarcoma; S-45 – animals with sarcoma 45.

essential to study those of the malignant human tumors removed at operation. The results are shown in the table.

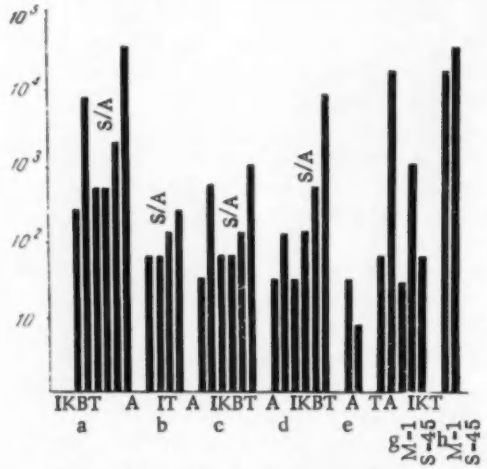

Fig. 2. Thrombokinase content of different tissues, expressed as the logarithm of the dilution of the tissue extracts. Indications as in Fig. 1.

As can be seen from the results, extracts of tumor tissue had the property of greatly reducing the clotting time of the plasma, and in most cases their thrombokinase activity was greater than that of the surrounding apparently normal tissue.

Our work has shown a definite similarity between changes in thrombokinase activity of tissues in endocrine disturbances and in tumors.

SUMMARY

Experiments were performed on white rats with various endocrine disturbances. Following adrenalectomy there was a considerable rise of thrombokinase activity of the lungs, kidneys, and brain. There was also an increased activity of the same organs after castration. In rats with tumor strains of sarcoma M-1 and sarcoma 45, there was a sharp rise in thrombokinase activity, mainly in the tumor tissue itself; the concentration of thrombokinase in the tumor tissue approached the highest concentration found elsewhere which was in the pulmonary tissue of castrated and adrenalectomized animals.

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BLOOD CLOTTING IN VITAMIN C DEFICIENCY. II.

PROTHROMBIN AND THROMBOTROPIN CONCENTRATION CHANGES AND THE REASONS FOR THE REDUCED BLOOD CLOTTING IN VITAMIN C DEFICIENCY IN GUINEA PIGS

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(Presented by Active Member of the AMN SSSR S. E. Severin)
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At present, nothing is known of the cause of the bleeding and petechial hemorrhages occurring in scurvy. The general opinion [5, 8, 12] is that the hemorrhage is due to an increased permeability of the capillary walls. Others [14, 15] consider that lack of vitamin C causes a disturbance of the blood clotting mechanism and that there is a fall in the amount of prothrombin which slows the clotting process. However, this has frequently been denied [16].

In previous experiments [1] we have shown that when guinea pigs are fed on a vitamin C deficient diet, besides the vitamin C deficiency, there are also blood changes in which the blood clotting rate is 30–40% below normal. It may be restored by daily injections of 2.5 mg of ascorbic acid. It has been shown that the effect on blood clotting is specific.

In the present work, a study has been made of the reduced clotting power of the blood in vitamin C deficiency, and prothrombin and thrombotropin have also been investigated.

METHOD

The experiments were carried out on guinea pigs weighing 300–370 g maintained on a vitamin C deficient diet consisting of rye straw, oats, oatmeal and water. After 12–15 days there was a fall in weight, and the animals died after 18–22 days. Post mortem examination revealed hemorrhages in the knee joints and ribs and the teeth were easily removed. Control animals on the same diet receiving daily injections of vitamin C gained weight and developed no symptoms. Samples of venous blood were collected from the jugular vein before, and at set times after being put on a vitamin C deficient diet, and changes in the concentration of the thrombogenic components, and in clotting times were determined.

The blood-clotting power was measured by the method of B. A. Kudryashov and P. D. Ulitina [7] as modified by us for guinea pigs [1]. Prothrombin concentration was measured by the method of B. A. Kudryashov, P. D. Ulitina and A. A. Pugacheva [6], and thrombotropin by B. A. Kudryashov's method [5].

Because the above methods were worked out on blood and thrombokinase taken from white rats, the clotting time of 80 normal guinea pigs was determined for different prothrombin and thrombotropin concentrations. The latter were varied by diluting the plasma with physiological saline. The thrombokinase was prepared from guinea pig brain tissue by a method described previously [6]. The results are shown in Fig. 1,a,b. The activity of the prothrombokinase of the blood platelets was determined by a method described by T.M. Kalishevskaya in which the platelets are separated by fractional centrifugation, and the prothrombokinase extracted with cold physiological saline. When activated by plasma thrombotropin active thrombokinase is formed which in the presence of $CaCl_2$ causes the plasma of normal animals to clot in 12–13 sec. The blood-platelet count was made by Fonio's method.

RESULTS

The results in Table 1 show that 13–15 days after the start of the experiment besides a fall in the clotting rate there is also a reduction in prothrombin and thrombotropin concentrations. There is less change in these concentrations than in the clotting rate, and the thrombotropin level falls by 38–35%. This result could hardly account for the fall in blood clotting rate of 62–61% (experiments 14 and 22).

Knowing that the blood clotting rate depends on the concentration both of thrombotropin and of prothrombokinase or platelet factor III [7], we have compared the levels of prothrombokinase in the blood platelets of experimental and control animals.

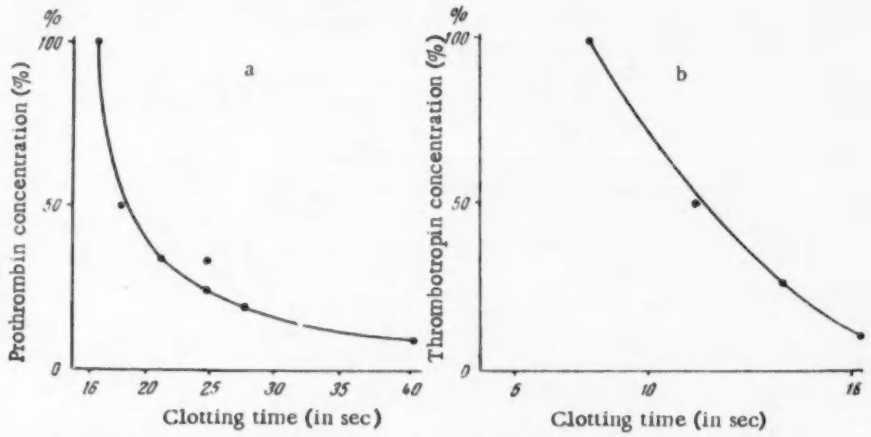

Fig. 1 Clotting time of normal guinea pig blood plasma. As related to (a) prothrombin concentration, and (b) thrombotropin concentration.

TABLE 1. Change in the Concentration of Prothrombin, Thrombotropin, and Clotting Rate in Vitamin C Deficient Guinea Pigs

No. of Experiment	Group of animals	No. of animals	Mean concentration in %								
			before experiment			after 13 -- 15 days			after 20 -- 21 days		
			prothrombin	thrombotropin	blood clotting rate	prothrombin	thrombotropin	blood clotting rate	prothrombin	thrombotropin	blood clotting rate
10	Experimental	5	91	92	100	61	65	58	67	75	21
	Control	3	90	90	100	84	96	90	100	100	98
14	Experimental	3	92	100	100	59	62	39	76[1]		44[1]
	Control	2	88	100	100	75	80	86	100		93
22	Experimental	5	88		93	69		38	58		38
	Control	5	90		95	87		100	100		100
Mean	Experimental	13	89	95	97	62	64	50	68	75	32
	Control	10	84	94	97	84	86	95	100	100	97

[1] Results from the one surviving animal.

The results of these experiments, given in Table 2 and Fig. 2, show that prothrombokinase activity is greatly reduced in the experimental group. After activating the platelet extract with thrombotropin from the blood of normal animals, in the presence of calcium chloride the active thrombokinase formed clots of normal plasma in 24 – 25 sec, i.e. it takes 10 – 11 sec longer than does thrombokinase obtained from normal platelets. To determine the difference in the activity (in sec), an activated platelet extract from normal animals was diluted in physiological saline until the activity was the same as that of the control group. It was found that a three- to fourfold dilution was required.

Figure 2 shows clearly the relationship between blood-clotting rate and the activity of the thrombokinase from the platelet extract. For 19 days, while the daily injections of ascorbic acid were given, both quantities remained at the normal level, but when the injection ceased there was at first a drop in blood clotting rate, and then a reduction in the activity of the thrombokinase obtained after activation of the platelet extracts of prothrombokinase by thrombotropin.

TABLE 2. Changes in Blood Clotting Rate and Thrombokinase Activity of the Blood Platelets in Vitamin C Deficient Guinea Pigs

No. of Experiment	Group of animals	No. of animals in group	Before the experiment				After 15 days			
			blood clotting rate (%)	clotting time of control plasma with calcium added			blood clotting rate (%)	clotting time of control plasma with added calcium		
					+ platelet extract	+ activated extract			+ extract of platelet	+ activated extract
21	Experimental . .	6	98	59	43	14	53	55	39	25
	Control . .	6	98	58	45	14	92	55	38	14
22	Experimental . .	5	93	55	39	14	38	52	33	24,5
	Control . . .	5	95	54	36	13	100	53	36	12

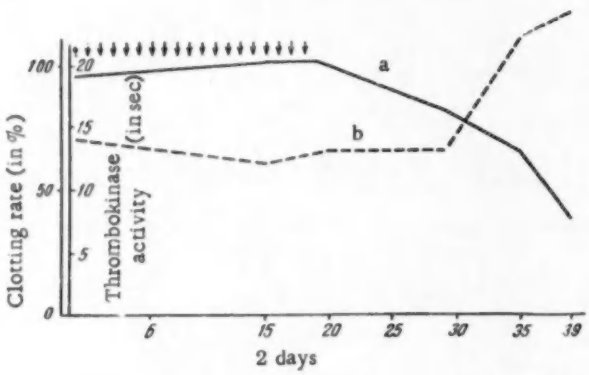

Fig. 2. Changes in blood clotting rate and prothrombokinase activity of the platelets in guinea pigs as affected by vitamin C. a – Clotting rate (in %); b – activity of thrombokinase obtained from platelets. ( ) shows moment of parenteral injection of 2.5 mg of ascorbic acid.1) Clotting rate (in %); 2) 2 days;3) thrombokinase activity (in sec).

It remains to be found whether the reason for the reduction in the prothrombokinase of the vitamin C deficient animals was due to a fall in the number of thrombocytes, or whether there was a qualitative change with a fall in their prothrombokinase content.

It can be seen from Table 3 that the number of platelets is reduced in vitamin C deficiency, and that this fall results in a reduced amount of prothrombokinase in the blood stream.

The results of these experiments show that one of the principal reasons for hemorrhages in vitamin C deficiency is lack of thrombokinase, which reduces the clotting power of the blood. Thrombokinase activity may be reduced on account of a disturbance in the synthesis of thrombotropin, or by lack of prothrombokinase in the platelets. Because the reduction in the concentration of thrombotropin is comparatively small, the fall in the thrombokinase activity is probably mainly due to a reduction in the amount of prothrombin, which in turn is partly caused by the considerable reduction in the number of platelets. Our results have been confirmed by others [3, 10, 14, 15]. There is also evidence [2] that ascorbic acid injections increase the number of platelets. However, the reduction in the activity in the thrombokinase obtained from the platelets of the experimental animals cannot be ascribed to the reduction in the number of the platelets themselves. The thrombokinase activity is reduced by a factor of at least three to four, while the number of the platelets is reduced by only a factor of two. It is probable that besides reducing platelet numbers, vitamin C deficiency also causes qualitative changes, and a reduction in the amount of thrombokinase per platelet.

The reduction in the prothrombin and thrombotropin levels is evidently the effect of vitamin C deficiency interfering with the synthesis of the substances. Both proteins are synthesized by liver cells. Ascorbic acid exerts a marked influence on the power of the liver to form protein [10]. In vitamin C deficient animals there is a marked fall in the protein concentration of the plasma, as well as a change in the relative amounts of proteins [14]. McCraw [14] found a reduction of approximately 50% in prothrombin concentration and attributed it to secondary vitamin K deficiency following lack of vitamin C. This explanation is well founded, because in the absence of ascorbic acid there is a complete absence of certain bacteria from the gut, including certain strains of Bacillus coli and B. paratyphus which are probably concerned in the synthesis of vitamin K. Probably the reduction in prothrombin and thrombotropin is not only caused by interference with their synthesis in the liver, but is also a secondary effect due to vitamin K deficiency.

TABLE 3. Change in the Blood Clotting Rate, Thrombokinase Activity, and No. of Blood Platelets in Vitamin C Deficient Guinea Pigs

No. of experiment	No. of animals	Before experiment			After seventeen days		
		blood clotting rate (in %)	mean thrombokinase activity (in sec)	no. of platelets per ml of blood	blood clotting rate (in %)	mean thrombokinase activity (in sec)	no. of platelets per ml of blood
22	4	100	13	487 931	61	23	235 640

SUMMARY

Vitamin C deficiency in guinea pigs causes a disturbance of the blood-clotting mechanism due to reduction of prothrombin, thrombin, and thrombokinase concentrations. The decreased amount of thrombotropin and the reduced thrombokinase content of the platelets reduce the amount of thrombokinase with the result that the clotting time is increased. The reduction in the amount of prothrombokinase is due to both a thrombocytopenia and changes in the platelets.

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THE INVOLVEMENT OF THE NUCLEAR SUBSTANCE OF ERYTHROCYTES IN EXPERIMENTAL HEMOLYTIC ANEMIAS

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There is no agreement concerning the nature of the degenerate remains found in erythrocytes lysed by various hemolytic agents. The fact that only mature erythrocytes are damaged, no degenerate structures are found in the young red cells, and the Feulgen reaction is negative have led Ya. Ya. Sokolovskaya [7, 8] to think that they result from injury and coagulation of the hemoglobin. E. I. Freifel'd [9] maintains that these structures are of nuclear origin. The fact that the hemolytic poisons do not affect the reticulocytes [9] is explained as being due to the presence of a basophil substance which prevents the degenerative structures from forming. There are other views, intermediate between those expressed above, concerning the origin of these structures.

Nevertheless, this problem is of considerable importance because an explanation of the nature of the degenerate structures may give information concerning their relation to certain preformed substances or to substances developing under the influence of the poison, and, in this way, may lead to a deeper understanding of the process whereby the erythroblasts lose their nuclei to form normal erythrocytes.

To study the nature of the degenerate structures, we investigated whether the hemolytic poisons affect the erythroblast nucleus and nuclear remains of young erythrocytes, which are stained by the hemolytic stain Azur II-eosin of D. I. Goldberg's method [1] and give a positive Feulgen reaction.

METHOD

We used our own method [4, 5] of a differential supravital stain for the internal structures of the erythrocyte. It differentiates the degenerative structures, which are stained green by brilliant green, from all the remaining regenerating structures which are stained red by neutral red. It was therefore possible to distinguish the numerous granular degenerate remains obtained from erythrocytes under the action of phenylhydrazine from the granular and thread-like substance of the reticulocytes, and also the Heinz bodies from the remains of the basophil cytoplasm and Howell bodies. By this method we have been able to reveal degenerate structures in cells where control smears treated with a single stain would have shown only the granular and fibrillar substance of the reticulocytes (Figs. 1 and 2).

To obtain degenerate structures in red cells, hemolysins (phenylhydrazine, aniline, benzidine, methylene blue) were injected subcutaneously. In all 33 mice, 14 rabbits, 5 guinea pigs, 6 frogs, and 3 pigeons were investigated. According to the particular poison used and the particular study being made, blood for a smear was taken every few minutes, or hours, or daily, or every other day, or sometimes less frequently. The smears were stained supravitally both by the differential method and by Nile blue (as a control) at intervals during the whole of the time from the beginning of the experiment until all red cells containing any remains had disappeared from the circulating blood. At the same time, smears were also prepared fixed in methyl alcohol and stained in Azur II-eosin.

To show the genetic relationship between the degenerate structures in the reticulocytes and the nuclear structures, fresh and supravitally stained smears were counterstained with the hemolytic stain Azur II-eosin after first fixing by the "time" method of D.I. Goldberg [1]. The Feulgen reaction was also performed for the same purpose. In rabbits, the same methods were used to study smears made from bone marrow punctures of the tibia and calcaneum.

Most of the experiments were carried out using phenylhydrazine, the strongest and most rapidly acting hemolytic agent whose action on the red cell has been thoroughly studied [2, 3, 6 - 8].

RESULTS

A few minutes after injecting the rabbits or mice with 50 mg/kg of aqueous phenylhydrazine, a fine granularity differentially stained green appeared in the erythrocytes. In the mature cells, most of the grains lay in groups, and sometimes in more or less massive conglomerates which sometimes formed a reticular structure. The resemblance of these erythrocytes to reticulocytes was so great that they could be distinguished from each other on the smear only by differential supravital staining. We were able to show that, contrary to the usual opinion, the hemolytic poison damages the reticulo-

cytes of the circulating blood. When it is injected into an animal with a normal number of reticulocytes the granularity representing degeneration which was found in them resembled that found in mature erythrocytes, but was coarser and stained an intense green color.

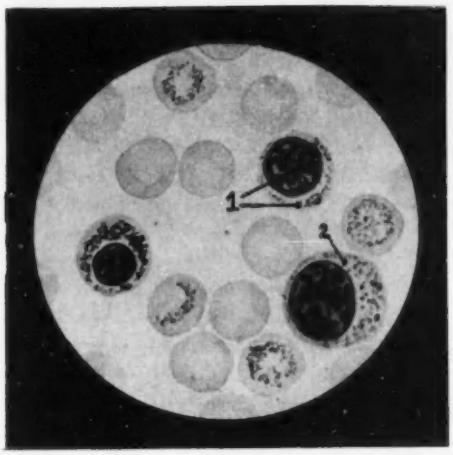

Fig. 1. Picture of bone marrow of rabbit before injecting poison. Differential supravital stain neutral red – brilliant green. Nuclei of all cellular elements and the granular and fibrillar substance are stained by neutral red. N.N.V. Objective.

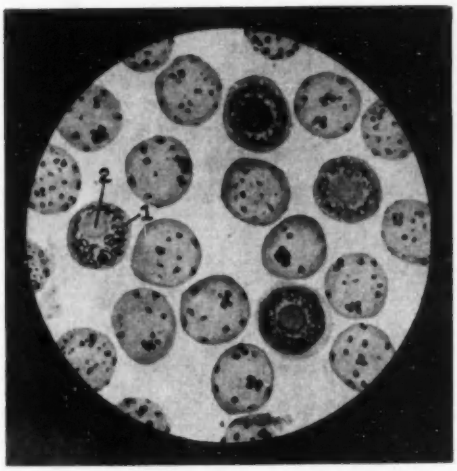

Fig. 2. Blood stained differentially with the supravital stain neutral red – brilliant green two days after poisoning the mouse with phenylhydrazine. The granulofibrillar substance continues to be stained by neutral red, while the necrotic nuclei and the granules which have degenerated are stained by brilliant green. N. N. V. Objective.

Original color plates were not reproduced due to technical difficulties. 1) Stains red; 2) Stains green – Publisher's note.

By frequent bleeding or repeated injection of the hemolytic agent, we stimulated the bone marrow strongly as was shown by the appearance of a large number of young forms of reticulocytes having an abundance of the granulofibrillar substance. When these cells are acted on by the hemolytic agent, reticulocytes with single large or numerous small homogeneous green bodies appear. The number of these cells is greater the stronger the stimulus to the erythropoietic system. As regeneration proceeds more rapidly, the degenerative structures found in the reticulocytes become larger. Then quite often reticulocytes are found with large degenerate structures in the center of the cell, where their roundness and the sharpness of their outline make them resemble an erythroblast nucleus (Fig. 2). Five to eight such cells per field of view were found in circulating blood, chiefly on the second or third day after injecting the hemolytic agent. The nuclei of the leucocytes did not change their color after the injection, and as before were stained red with the differential stain. There was no change in the morphological features of the degenerate granules which was related to the amount of the injected hemolytic agent. Repeated injections caused a change only in the number of unaffected reticulocytes.

Wheras mature erythrocytes with degenerate granules were found in circulating blood over a period of 8 – 10 days or more, reticulocytes with such granules survived for a time which was shorter the younger the cell; when only reticulocytes were present and hemolytic agent was injected, the damaged cells were replaced by new reticulocytes from the bone marrow in 2-3 days.

During the development of phenylhydrazine anemia, when there are a large number of reticulocytes, erythroblasts are almost absent from the circulating blood. Occasional cells were found which were stained by Azur II-eosin and which had homogeneous disk-shaped nuclei which did not stain cherry red, but blue or pale blue.

These results led us to study hemopoiesis in phenylhydrazine anemia. Bone-marrow punctures were examined at different time intervals after injecting a hemolytic agent, but no degenerate structures could be observed in the protoplasm of erythroblasts, though there were changes in their nuclei. Some of the nuclei, instead of staining bright red began to show a green shade and the result of the mixture of the two colors was to produce a diffuse brown spot. In some nuclei which were stained red, there were isolated bright green areas. After some hours, these cells lost their resemblance to erythroblasts, because their nuclei took up the green stain more strongly, as though changing into the usual disk-shaped reticulocyte bodies.

A careful study of the damaged cells showed that the rate at which the degenerate structures appeared in the erythrocytes after the injection depended on their age; the first to be affected were the erythrocytes and the more mature reticulocytes, and then the more mature erythroblasts. This feature is shown more clearly when a

more slowly acting agent is used (aniline, methylene blue, nitrobenzene).

In spite of repeated injections of large does of phenylhydrazine, the nucleated red cells of frogs and pigeons showed no degenerate structures in the protoplasm. However, after the injection green granules could be revealed by differential staining. Sometimes all the nuclei stained brown. In some erythrocytes, the nucleus was not clearly outlined.

The resemblance between the degenerate and the Azurophil structures in the reticulocytes, and particularly the relationship between their appearances and the degree of maturity of the cell, led us to investigate the relationship between the structures. The nuclei of the erythroblasts and the Azurophil structures gave a positive Feulgen reaction, but the later the time after the injection the less clearly did these structures appear, and after 2 – 3 hours from the time of the injection they could not be made out, even when before the injection they had appeared in each cell.

We have concluded that normally the nucleus of an erythroblast becomes lost by becoming gradually more dense, and at the same time passing into solution. The Azurophil nuclear remnants are detected at first by using an hemolytic stain and Feulgen's reaction, when they appear in the young erythrocyte as coarse threads, clumps, and granules, whereas in the more mature cells the granularity no longer occurs. Hemolytic agents cause many intracellular changes and bring about the swelling and the change in adsorptive properties of the nuclear fragments, so that they are no longer shown up by Feulgen's test for nucleic acid, though they do appear as degenerate structures when smears are stained supravitally. Degeneration of the substance of the nucleus takes place more rapidly in mature cells than in young reticulocytes or in erythroblasts. The fact that after the degenerate structures have been formed the cell dies more quickly the younger it is suggests that the irreversible process by which the nuclear substance forms degenerate products leads to the loss of some substrate, whose function is of greater importance the younger the cell.

Our experiments have shown that the hemolytic agents, and particularly phenylhydrazine, have a more widespread action than was previously supposed. Besides destroying hemoglobin they also induce necrotic changes in erythroblast nuclei and in Azurophil nuclear remnants, and in this way cause the formation of the degenerate structures of nuclear origin.

SUMMARY

A study was made of the effect of hemolytic agents (phenylhydrazine and aniline) on the nuclei of erythroblasts and the nuclear remnants of young erythrocytes in mice, rabbits, guinea pigs, frogs, and pigeons. The author's own method of supravital staining with neutral red – brilliant green was used, as well as D. I. Goldberg's method for smears, and Feulgen's reaction. It was shown that hemolytic poisons act on erythroblast nuclei and erythrocytic nuclear remnants and assist the formation of degenerate nuclear structures.

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THE EFFECT OF EXPERIMENTAL DYSENTERY INTOXICATION ON UREA PRODUCTION IN RATS

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It is well known from clinical practice that dysentery is often accompanied by a change in the functional state of the liver [6, 12]; a disturbance of the antitoxic function of the liver has also been noted [3, 7, 8, 13], but of course, the liver function test for hippuric acid synthesis, ordinarily used in the clinic, does not completely reflect the states of the detoxicating function of the liver. It was therefore of interest to study the effect of dysentery intoxication on other indices of liver function.

Data have been published [8] concerning the effect of dysentery toxin on the capacity of the liver to conjugate phenols, but we have not found any information about the effect of this toxin on so important a function as urea production. When we consider that this process undergoes significant disturbances under other types of toxic influences, such as radiation sickness, prolonged medication sleep, thyrotoxicosis, and exposure to radiation in the far ultraviolet [1, 4, 10, 11], it is to be expected that bacterial toxins will exert a marked effect on it.

In the present study we examined the blood urea content and urea production by liver slices in experimental dysentery intoxication in white rats.

METHOD

The model of dysentery intoxication described by V. I. Mchedlishvili [5] was employed. Complete Flexner's dysentery antigen, obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology, was injected intramuscularly into white mice weighing 120 – 130 g in a dose sufficient to produce death in 60 – 70% of the animals within three to four days. The animals were examined 24 hours after injection of the antigen. In all, 50 rats were used.

For the blood urea determination the animals were decapitated, 1 ml of blood was placed in a test tube with 1 ml of 20% trichloroacetic acid, and the supernatant fluid was obtained by centrifugation. Urea was determined by the urease method. Soybean extract was used as urease. The urea concentration was expressed as mg% nitrogen.

For the determination of urea production in liver slices, the liver was rapidly removed, slices were prepared in the cold, and after two rinsings slices weighing about 400 mg were placed in Krebs–Ringer + bicarbonate buffer (pH 7.4). NH_4Cl was added to a final concentration of M/100 as an ammonia source, and the slices were saturated for 10 minutes with a mixture of 95% oxygen and 5% CO_2, and then incubated for two hours at 37° with shaking. Proteins were precipitated with trichloroacetic acid, and free ammonia (by Conway's method) and urea were determined in the filtrate. The amount of urea synthesized during incubation was obtained from the difference between the nitrogen concentrations before and after hydrolysis with urease. The urea concentration was expressed in μM nitrogen per gram of wet tissue.

RESULTS

Twenty-four hours after injection of diphtheria antigen, intoxication was outwardly manifested, in the majority of the rats, in a significant reduction of overall activity and activity related to eating, ruffling of hair, dyspnea, and other symptoms. Individual animals were in extremely poor condition with clear-cut signs of impending death (lying on their sides, absence of reaction to external stimuli). Such animals were not used in the experiments with liver slices, but a blood urea determination was made on some of them. They were placed in a separate group.

The results of the blood urea determinations on control rats and rats examined 24 hours after injection of dysentery antigen are shown in Table 1.

It is evident from the data in Table 1 that the mean urea content in the blood of experimental animals was less than that of control animals (control, 16.3 mg%); experimental, 13.2 mg%). The rats mentioned above, which were extremely ill when the blood was taken, were an exception. The urea concentration in the blood of these animals was several times as high as for normal animals (from 39.6 to 103.3 mg%). The condition of these animals should not be considered as resulting from the influence of dysentery toxin alone – it is certain

TABLE 1. Blood Urea Content in the Presence of Dysentery Intoxication, in mg% Nitrogen

No.	Control animals	Animals with dysentery intoxication	
		moderately ill	extremely ill
1	19,0	12,0	85,7
2	10,4	7,8	52,2
3	16,5	11,4	103,3
4	17,2	11,4	39,6
5	17,7	17,4	54,0
6	11,0	14,4	65,4
7	18,9	16,2	—
8	18,3	13,8	—
9	13,8	16,8	—
10	19,8	10,4	—
Mean	16,3±1,06	13,2±1',06	

TABLE 2. Urea Synthesis in Rat Liver Slices in the Presence of Dysentery Intoxication.

No.	Urea content, in μM nitrogen per gram wet tissue	
	controls	animals in a toxic condition
1	65	46
2	56	38
3	57	59
4	45	26
5	41	28
6	44	22
7	67	54
8	77	54
9	69	37
10	49	53
11	45	60
12	—	61
13	—	38
14	—	46
Mean	56±3,7	44±3,5

that other disturbances are also occurring which are characteristic of the agonal state [2]; consequently, the high urea values obtained for these animals probably should be attributed to a disturbance in renal activity, and impairment of urea excretion by the organism.

In the main group of experimental animals, with the usual syndrome of dysentery intoxication, we observed that the blood urea content was lower than in the controls, which is evidence that a disturbance of liver activity had occurred. To ascertain the cause of the reduction in blood urea content, we conducted experiments with liver slices. The results of these experiments are set forth in Table 2.

It is clear from the data of Table 2 that liver slices from normal rats synthesize, on the average, 56 μM urea nitrogen in two hours while the corresponding figure for experimental animals is 44 μM nitrogen (a 22% reduction).

The reduction in urea synthesis by liver slices from experimental animals indicates that dysentery toxin has a depressant effect on urea production by the liver. In the intact organism this is manifested in a reduced blood urea concentration.

Our data thus provide additional material to characterize the effect of dysentery toxin on liver function. In addition to producing a disturbance in phenol conjugation [8] and hippuric acid synthesis [9], this toxin markedly depresses urea production.

SUMMARY

This paper deals with the effect of dysentery intoxication on one detoxifying function of the liver – urea formation – in rats. The experiments showed that the blood urea level in experimental animals is somewhat reduced in comparison with control animals, 24 hours after administration of Flexner's dysentery antigen. Urea synthesis in liver slices from these animals is also reduced, which points to a depressing effect of dysentery antigen on this process.

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*Original Russian pagination. See English translation.

THE COURSE OF EXPERIMENTAL TETANUS INTOXICATION IN ALTERED FUNCTIONAL STATES OF THE CENTRAL NERVOUS SYSTEM

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Among possible experimental approaches to the development of an effective tetanus treatment, an important and promising approach is to explore the possibility of regulating the relation between excitation and inhibition in the cerebral cortex at various stages of the tetanus process.

The significance of inhibition (primarily medicinal and anesthetic) in experimental tetanus has already been examined [4, 8, 10, 11, 13, 16, 19, etc.], but in view of discrepancies in the results the published data do not allow definitive conclusions and necessitate further investigations of the role of strengthening the excitatory and inhibitory processes in the cerebral cortex in tetanus in order to indicate, as far as possible, the future direction of research toward effective treatment of this disease. In studying the role of cortical processes, particularly inhibition, in the development of tetanus intoxication, it is desirable to eliminate such negative properties of hypnotics and general anesthetics as their toxicity, their different effects on different portions of the central nervous system, and their rapid elimination from the organism; in all probability, the last mentioned is accompanied by a change in the relative strength of excitation and inhibition in the cerebral hemispheres, and clearly causes the lack of agreement among published reports.

Accordingly, we thought it would be best to use the method of strengthening inhibition and reducing cortical tone without anaesthetic by "deafferentation" – accomplished by excluding the receptor terminations of the olfactory, visual, and auditory analyzers [1, 2, 3, 7, 9, 12, 15, 18, etc.] – and also by partial decortication of the brain. At the same time, we decided to test the effect of amphetamine sulfate, which strengthens the stimulatory process, stimulates central nervous system activity, and adjusts disturbed relationships between inhibition and excitation in the cerebral cortex [5, 17, etc.].

During the period when these experiments were conducted (1951 – 1956), we were unable to find any indications in the available literature that experimental tetanus had been studied under conditions of exclusion of the olfactory, visual, and auditory analyzers, or administration of amphetamine sulfate.

METHODS AND RESULTS

We performed the first series of experiments on 92 rabbits (47 with excluded olfactory, visual, and auditory analyzers and 45 controls with uninjured analyzers) divided into groups according to the length of time following exclusion of the three distance analyzers and the dose of tetanus toxin. Tetanus toxin was administered to all rabbits via the muscles of the left hip, 6, 29 – 35, and 122 – 183 days after exclusion of the visual and auditory analyzers *.

The results of these experiments showed that after injection of 1 MLD or 1/10 MLD of tetanus toxin at a late period (29th to 35th day, 122nd – 183rd day) or at the earliest period (6th day) after the second step in "deafferentation" (vision and hearing), a distinct retardation of the symptoms of the disease was observed in the first few days of the experiment in all the animals with excluded analyzers as compared with the controls. This is in agreement with published data [4, 11, 14, etc.]. The indicated authors observed that during the first period of development of tetanus intoxication, manifestations of the initial symptom complex of tetanus were absent in animals under amobarbital, chloral hydrate, or ether anaesthesia. We may conclude from this that the inhibitory

* The analyzers were excluded in stages. The olfactory analyzer was excluded first by sectioning and removing the olfactory bulbs; ten days later, the visual and auditory analyzers were excluded by suturing together three tissue layers (rudimentary eyelid, conjunctiva, and skin of eyelid) over the uninjured eyeball, preserving the optic nerve and normal circulation of lacrimal fluid. To exclude the auditory analyzer the tympanic membrane and the auditory ossicles were injured and removed, and the muscles and skin were then sewn in successive layers to the external orifice of the bony portion of the auditory canal.

TABLE 1. Effect of Exclusion of Three Distance Analyzers on Survival Time of Rabbits After Injection of 1 MLD of Tetanus Toxin

Animals	Total number	Number dying in given period of observation			Mean survival time (days)
		1–5 days	6–20 days	after 20th day	
With excluded analyzers . . .	23	14	9	—	6,5
Controls	23	5	16	2	8,7[1]

[1]Mean of observations on 21 rabbits.

process, strengthened in the central nervous system of animals not only by medicinal agents but also by a disturbance of the function of the three distance analyzers, exerts the same retarding influence on the development of the tetanus snydrome in the initial period of the disease.

Further observations established that, as the pathological process develops, marked worsening of the disease and earlier death occurred at the stage of generalization of tetanus in rabbits with excluded analyzers.

Table 1 shows that by the fifth day following injection of 1 MLD of tetanus toxin, 14 out of 23 animals in the group with excluded analyzers had died, and in the control group five out of 23; the mean survival time of animals with excluded analyzers was less (6.5 days) than that of the controls (8.7 days). Injection of 1/10 MLD of toxin usually produced a mild, localized tetanus in normal, unoperated rabbits with subsequent rapid recovery. In "deafferented" animals (with considerably reduced afferentation, in consequence of exclusion of the three distance analyzers), as a result of the injection of the same dose of toxin, the course of intoxication was observed to be milder than in the case of the controls during the first days of the illness; but, later on, the localized tetanus was observed to pass over into a severe generalized form, terminating in death of the overwhelming majority of the "deafferented" animals.

Table 2 shows that following intramuscular injection of 1/10 MLD of tetanus toxin, of 24 rabbits with excluded analyzers only 11 recovered and 13 died of generalized tetanus. In the control group, out of 22 animals only two died, and 20 recovered. The course of tetanus intoxication following injection of 1 MLD and 1/10 MLD of tetanus toxin in animals with excluded distance analyzers was observed to be more severe at 29–35 days after the second stage of "deafferentation" (exclusion of visual and auditory analyzers) than at six days and 122–183 days. It seems to us that this eliminates any question of the significance of operative trauma in the process under consideration.

Thus our data show that prolonged strengthening of inhibition in the cerebral cortex of animals, during the later period of development of tetanus intoxication, exerts a negative effect on the course and outcome of the pathological process. This is in agreement with data in the literature [14, 16, 19].

In our opinion, this conclusion is supported by experiments performed by us on 66 rabbits with ablation of the upper surface of the left cerebral cortex (from the olfactory bulb to the posterior margin of the occipital lobe), and on 63 control (unoperated) rabbits, divided into groups on the basis of postoperative period (6–8, 22–35, 175–186 days), site of injection of the toxin (muscles of the right or left hip), and dose of tetanus toxin (1 and 1/19 MLD).

Experiments conducted under these new conditions of reduced cortical compensatory potentialities showed that the largest effect of decortication on the course and outcome of the tetanus process in rabbits decorticate on the left side is usually noted when they are injected in the limb innervated by the decorticate hemisphere. In such cases, the animals are not only more severely affected with tetanus than the controls, but are also more affected than animals similarly decorticate but receiving the toxin in the opposite limb; they die considerably earlier or recover later.

Thus, whereas after injection of 1 MLD of toxin in the muscles of the right hip rabbits decorticate on the left side die, on the average, after 7.5 days, the corresponding controls die after 11.6–12.4 days and decorticate animals given the same dose of toxin in the muscles of the left hip died after 9.9 days.

Rabbits decorticate on the left side were observed to recover from the injection of 1/10 MLD of tetanus toxin in the muscles of the right hip, on the average, after 32.3 days. Recovery of similarly decorticate animals, injected with the same dose of toxin in the muscles of the left hip, was noted after an average of 22.6 days. Control rabbits that received the same dose of toxin recovered after an average of 20.6–18.7 days.

To generalize from the results of these two series of experiments, we may conclude that strengthening of inhibition and reduction of the compensatory capabilities of the cortex and the work capacity of cortical cells has an aggravating influence on the outcome of tetanus intoxication. This idea gave rise to the supposition that during tetanus, at a certain stage in its development, it would be useful to administer substances that stimulate central nervous system activity. For this purpose we have been testing amphetamine sulfate since 1953.

In the experiments with amphetamine sulfate more than 100 rabbits were used, divided into five groups; these received intramuscular injections of 1, 2, 3, and 12 MLD of tetanus toxin. The experimental animals received amphetamine sulfate intramuscularly in a dose of 1 mg per kg weight, as a rule, at the stage of generalization of the pathological process.

The results of the observations are presented in Table 3. Amphetamine sulfate injected at the stage of generalization of the tetanus syndrome exerted a favor-

TABLE 2. Severity of Tetanus in Rabbits With Excluded Analyzers during the Second Period after Injection of 1/10 MLD of Tetanus Toxin

Animals	Total number	Severity of tetanus process					Number of animals	
		local			generalized		recovered	dead
		mild	moderate	severe	mild	severe		
With excluded analyzers	24	1	6	2	5	10	11	13
Controls	22	7	3	7	5	—	20	2

TABLE 3. Effect of Amphetamine Sulfate on Survival Time of Rabbits Given Various Doses of Tetanus Toxin

Doxe of toxin (MLD's)	Experimental group				Control group	
	experimental group	time of injection of amphetamine sulfate after injection of tetanus toxin	stage of development of tetanus intoxication	mean survival time (days)	number of animals	mean survival time (days)
1	7	Simultaneously with injection, and 24 hrs. after injection	Absence of disease symptoms, localized tetanus	17,0	12	17,5
	4	Six days after injection	Generalized tetanus	40,2		
2	10	4 days	The same	33,5	11	15,1
3	12	3 days	» »	7,1[1]	14	5,7
12	6	2 days	» »	4,8	7	3,3

[1]Excluding one rabbit that was sacrificed on the 84th day after injection of toxin.

able effect on the course of tetanus; the experimental animals lived longer than the controls.

We also observed a favorable effect of amphetamine sulfate therapy when this preparation was injected three to six days after injection of the toxin. Injection of amphetamine sulfate either prior to injection of the animals with lethal doses of toxin, or simultaneously with such injection, or 24 hours afterward had almost no effect. It should be added that combined amphetamine–serotheraphy of rabbits, which received serum and amphetamine sulfate beginning with the third or fourth day after injection of 2 MLD of tetanus toxin, was more effective than serotherapy without amphetamine.

The feature we have noted – that exclusion of the olfactory, visual, and auditory analyzers, as well as administration of amphetamine sulfate, has an effect on various stages of development of the tetanus process in rabbits – can apparently be explained, in our opinion, by an alteration of the functional state of the central nervous system in the course of the development of tetanus. In all probability this assumption is correct. It is supported, first, by the data of E. A. Gromova [6], who showed a change in the electroencephalogram of animals suffering from tetanus, which was characterized by predominance of the stimulatory process in the cerebral cortex at the stage of localized tetanus, and passed over into a predominance of inhibition as the disease became generalized; secondly, by the phenomenon we have observed, that the course of tetanus intoxication is either more severe or more favorable, depending on the stage of its development – probably because of summation of the functional states of the cerebral cortex that have been created (by exclusion of the distance analyzers and by administration of amphetamine sulfate to the animals) and altered in the course of tetanus.

SUMMARY

Tetanus intoxication was much more severe in rabbits with surgical ablation of elements of the left cerebral cortex and in rabbits with three distance analyzers

(olfactory, visual, and auditory) excluded than in controls. Stimulation of central nervous system activity by administration of amphetamine sulfate results in increased resistance to tetanus and prolonged survival time.

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AN ELECTROPHORETIC AND IMMUNOCHEMICAL STUDY OF SERUM PROTEINS IN GUINEA PIGS DURING DEVELOPMENT OF BRUCELLOSIS

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The prolonged complement-fixation reaction (PCFR) in the cold is one of the most discriminative and sensitive methods of investigation. With the help of this reaction it is possible to demonstrate both antibodies and antigens in material under study.

E. S. Orlov and S. D. Skul'skaya [5], M. L. Feder [8], P. A. Trilenko [6], and T. M. Torosov [7] have used the PCFR in diagnosing brucellosis, and have given it a high rating. B. I. Ioffe [3] suggested that in the demonstration of specific antigen in blood sera of guinea pigs, the antigen should be concentrated by passing streams of carbon dioxide through the serum. Our observations on experimental brucellosis in guinea pigs, with the use of this method, indicate that specific antigen can be demonstrated in serum even at early stages in the development of infection. Specificity of the precipitate obtained has also been demonstrated by allergy tests on sensitized guinea pigs, and by immunizing rabbits with this precipitate and subsequently determining specific antibodies in them.

The purpose of the present study was to conduct an immunochemical and electrophoretic investigation of blood sera of guinea pigs throughout the development and the entire course of the infectious process, before and after passing CO_2 through the sera. Preliminary experiments of ours showed that as a result of the passage of CO_2, considerable depletion of serum proteins occurs; these are evidently precipitated when the pH of the solution is altered toward the acid side. When CO_2 is passed through serum from healthy animals, a precipitate is likewise formed in some cases, but considerably less than in the case of infected guinea pigs, and it is practically impossible to analyze it electrophoretically.

METHODS

Guinea pigs were inoculated with a live culture of Brucella melitensis, in a dose of 1000 microbial cells. Prior to inoculation, and at a definite time thereafter, blood was drawn from each animal to obtain the serum. Serum was diluted 1:4 with distilled water, and carbon dioxide was passed through it for 10 – 15 minutes.

The passage of CO_2, which shifted the reation of the solution toward the acid side, resulted in precipitation of certain protein components from the serum, which were subjected to electrophoretic analysis after centrifugation and two rinsings with further centrifugations – along with the supernatant fluid, which was arbitrarily designated the "non-precipitable fraction" of the serum. Simultaneously, after the same time intervals, sera from healthy animals were subjected to electrophoretic analysis before and after CO_2 was passed through them.

In the whole serum and in the precipitate formed after the passage of CO_2, the protein content was determined by Lowry's method [10]. For electrophoretic analysis, an amount of serum was used which contained 300 – 500 γ of protein. After electrophoresis the individual protein fractions were determined by densitometry. The precipitate formed after the passage of CO_2 was rinsed twice with distilled water, for the purpose of ridding it of other serum proteins, and then dissolved in buffer, yielding a faintly opalescent solution, which was poured onto paper for electrophoresis. Electrophoresis was carried out in a barbital – barbital sodium buffer at pH 8.6, ionic strength 0.1, for 17 hours, with a potential of 175 volts and a current of 6 ma, on fast-absorbing chromatographic paper measuring 40 cm x 4 cm; the paper was subsequently stained for proteins, carbohydrates, and lipids. For immunochemical study of the precipitate, we used the methods of immunoelectrophoresis in gels, with the modifications of Kohn [9] and Gendon [1], with microprecipitation in a gel and precipitation on paper [2]. In addition, qualitative reactions were carried out for the detection of lipids (staining with Sudan black) and polysaccharides (staining by Hotchkiss's method, with basic fuchsin) [3] in lyophilized precipitate.

For a control in staining for lipids and polysaccarides, crystalline human albumin was used, which did not stain with Sudan black or with basic fuchsin, and lipids and

polysaccharides isolated from brucella and typhoid bacteria, which stained well with these dyes. Simultaneously, the possible presence of DNA was determined on the basis of absorption by the precipitate in the ultraviolet at a wave length of 270 – 280 Å, with absorption by a known nucleoprotein isolated from typhoid bacteria, and rabbit serum, which does not contain DNA, as controls.

At the same time as the chemical and immunochemical studies, whole sera and individual fractions (nonprecipitable fraction and precipitate) were subjected to immunological analysis for the demonstration of specific antigenic substance. For this purpose, the PCFR in the cold and an allergy test on sensitized guinea pigs were employed. In conducting the PCFR, and in all experiments of the chemical and immunochemical analysis, we used specific brucellosis serum with a titer of 1:6400; in experiments with diffusion microprecipitation in gels, the serum was diluted 10 – 20 times.

RESULTS

Table 1 shows the quantitative determination of protein fractions of blood serum at various periods after inoculation. The control was serum from a healthy guinea pig. In all cases, 300γ of protein was taken for electrophoresis. As is evident from this table, during the development of brucellosis infection in guinea pigs a considerable reduction takes place in the content of albumins (from 63.7% to 35.4%), along with an increase in the α- and γ-globulins, with irregular fluctuations in the β-globulins.

In Table 2, quantitative data are presented on the content of various protein components in the "nonprecipitable fraction" of blood serum of guinea pigs at various times after inoculation.

It is clear from this table that the principal regularities in the changes of serum proteins during the development of infection remain the same: The albumin content is reduced and the γ-globulin content increases. The content of α-globulins in the "nonprecipitable fraction" was significantly reduced after the passage of CO_2.

On the basis of these data we may assume that α-globulins are the most unstable to changes in the pH of the solution, and are precipitated when the serum is acidified with CO_2. We were able to demonstrate the correctness of this assumption after painstaking electrophoretic analysis of twice-washed and lyophilized precipitates obtained from the sera of animals at various intervals after the moment of inoculation. Since we had extremely limited quantities of precipitate at our disposal in this work, we could not approach the estimation of it in a strictly quantitative manner, and were forced to limit ourselves to a qualitative characterization. Figure 1 shows the electrophoretic chromatogram of a precipitate obtained from blood serum taken from a guinea pig 24 hours after inoculation with a brucella culture. By this time, significant quantitative changes have occurred in the protein composition of the serum. The same figure shows a curve of the quantitative relationships of the proteins detected, and the electrophoretic chromatogram of normal guinea pig serum. As can be seen, the principal protein component of the precipitate corresponds in position to the α-globulin fraction.

The least mobile component of the precipitate is distributed between the β- and γ-globulins. This demonstrates the correctness of our belief that when CO_2 is passed through guinea pig serum the α-globulins are precipitated from it. Although a reduction in albumin content occurs when infection develops, albumin was not detected in the precipitate*

As mentioned earlier, precipitates formed from the sera of infected guinea pigs show up in the prolonged complement-fixation reaction (PCFR) as antigens. It might be assumed that proteins precipitated from serum upon acidification with CO_2 adsorb antigens, or products of microbial metabolism having an antigenic action, on their surface. For this reason, it was of great interest to test the antigenic properties of precipitates by the latest immunochemical methods. Despite repeated efforts, using the methods of immunoelectrophoresis, microprecipitation on a microscope slide, and precipitation on paper, we were unable to demonstrate any antigenic properties of the precipitates. From this we concluded that either

* We wish to thank A. Ya. Fridenshtal' and M. Ya. Kern for assistance in analyzing precipitates for lipids, polysaccharides, and DNA.

TABLE 1. Electrophoretic Components of Whole Blood Sera of Guinea Pigs at Various Times After Inoculation

Time after inoculation	Amount present (%)			
	albumin	globulins		
		α-	β-	γ-
Normal	63,7	17,2	8,7	10,4
9 hours	66,1	22,0	2,6	10,3
12 »	50,1	21,7	12,2	16,6
48 »	42,2	16,9	7,0	33,9
120 »	43,1	31,3	5,9	19,6
240 »	37,8	32,8	7,0	22,4
360 »	35,4	36,2	6,8	24,7

TABLE 2. Electrophoretic Components of "Nonprecipitable Fraction" of Blood Sera of Guinea Pigs at Various Times After Inoculation

Time after inoculation	Amount present (%)			
	albumin	globulins		
		α-	β-	γ-
Normal	51,2	27,3	7,2	14,3
3 hours	50,0	31,0	6,0	14,0
12 hours	48,3	24,2	8,0	19,5
24 hours	50,5	29,0	7,0	14,6
48 hours	48,3	24,6	10,6	18,5
72 hours	43,0	18,0	11,0	28,0
120 hours	46,0	17,47	10,5	26,9

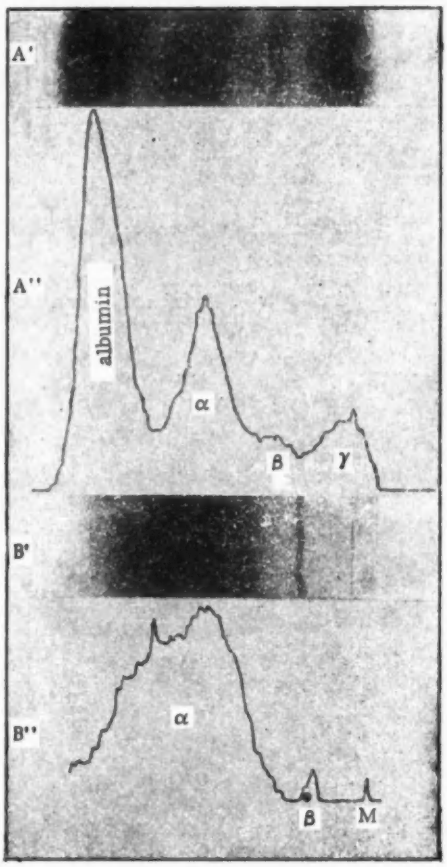

Fig. 1. Electrophoretic distribution of protein fractions of precipitate formed from blood serum of guinea pigs 24 hours after inoculation. A' – electrophoretic chromatogram of normal serum; A'' – densitometric curve; B' – electrophoretic chromatogram of precipitate; B'' – densitometric curve; M – spot marking infection.

all these methods were insufficiently precise and limited in resolving power, or else substances that have an antigenic action in the PCFR are in reality haptens and can not give typical antigenic reactions in immunochemical analyses.

The latter explanation seems probable, since experiments with allergy tests on sensitized guinea pigs, like the positive complement-fixation reaction, confirmed the presence of antigenic properties in precipitates formed upon the passage of CO_2, even at early stages after the beginning of infection.

Qualitative chemical analysis of the precipitates showed that these contain lipids and polysaccharides, but not DNA. The presence of lipids and polysaccharides may be connected with precipitation of microbial antigen as well as serum components. Experiments with differential staining of electrophoretic chromatograms for lipids and polysaccharides (Wunderly and Piller [12]) have shown that both classes of substances are present in all electrophoretic fractions of the sera studied.

On the basis of studies of ultraviolet absorption by precipitates from sera of infected guinea pigs, it was established that DNA is not contained in the precipitate.

To summarize the results of our investigations, therefore, we may conclude that in the process of development of brucellosis an increase in α-globulins and γ-globulins occurs in guinea pig serum, with a simultaneous reduction in albumin content; when CO_2 is passed through the serum at various times after the moment of inoculation, a certain fraction of the proteins is precipitated, which is similar in mobility to serum α- and β-globulins. A certain amount of lipid and polysaccharide material is also precipitated.

Biological methods (PCFR and allergy tests on sensitized guinea pigs) show that the precipitate has antigenic properties, but these could not be demonstrated by any of the physicochemical methods tried.

SUMMARY

It was shown by electrophoretic methods that in the early stages of brucellosis in guinea pigs, there is an increase in the α- and γ-globulin fractions of blood serum, and a reduction in the albumin fraction.

The α-globulin content is decreased in the "nonprecipitable fraction" left in solution after CO_2 is passed through the serum; electrophoretic analysis shows that the

γ-globulins are precipitated when the medium is acidified. The prolonged complement-fixation reaction, and allergy tests on sensitized guinea pigs, have demonstrated that the precipitate possesses antigenic properties undetectable by any of the physicochemical methods used The precipitate contained two groups of proteins whose mobility approached that of the α- and β-globulins, as well as lipid and polysaccharide material. The possibility is discussed that antigenic substances from brucella, or products of brucella metabolism, are adsorbed by serum protein components of high mobility.

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ENERGY METABOLISM OF PLATELETS

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Among the formed elements of the blood, the structure, composition, and metabolism of the thrombocytes (blood platelets) have been least studied. We need only point out that up to now it has not been conclusively established whether the platelets are cells or fragments of the cytoplasm of megakaryocytes, and that the question of whether a nucleus is present in these elements also has not been entirely settled. Negative results obtained in searching for desoxyribonucleic acid in platelets by various chemical and histochemical methods caused Maupin [2] to classify them as "incomplete" cells.

It is well known, however, that these formed elements are characterized by the presence of several enzymes [2], and play a substantial role in the process of blood coagulation.

As noted above, the metabolism of platelets has not been extensively studied. Tullis [3], who found only insignificant glucose and oxygen consumption and lactic acid accumulation, believes that platelets have little or no metabolic activity. Other authors, however, hold the opposite view [1].

METHOD AND RESULTS

Experiments were conducted on platelets removed from the blood of donors on the same day it was drawn, by the method of two-step centrifugation in a refrigerated centrifuge at +4°. For the prevention of agglutination and traumatization of platelets, a solution of Trilon B (the disodium salt of ethylenediaminetetraacetic acid) was added to the suspension. From 800,000 to 3,000,000 platelets were contained in 1 mm of suspension. The platelet suspension in plasma was of a high degree of purity.

The platelet suspension was studied on the day it was prepared, or the day following. Respiration was determined manometrically in a Warburg apparatus with the flasks saturated with oxygen; glycolysis, from the accumulation of lactic acid, the amount of which was determined colorimetrically with p-hydroxyphenyl; sugar, by the method of Hagedorn and Jensen, with cadmium reagent; and ATP, after an 8-minute hydrolysis of a trichloroacetic filtrate in 1N HCl, for inorganic phosphate, by the method of Fiske and Subbarow.

RESULTS

It was found that platelets consume oxygen and have the capacity for glycolysis. It is characteristic that, like leukocytes (granulocytes), platelets carry on glycolysis under both aerobic and anaerobic conditions, anaerobic glycolysis being considerably more intense than aerobic (Table 1).

The values cited are for 1 ml of densely packed platelets incubated for two hours. It is likewise characteristic that the addition of glucose caused a depression of oxygen consumption by the platelets (see figure).

A depression of respiration was observed following the addition of various amounts of glucose (Table 2).

The concentration of sugar in the platelets, and the consumption of sugar during incubation were small. When glucose was added to the platelet suspension, its consumption increased, especially under anaerobic conditions, in comparison with samples to which glucose was not added although not all the glucose in the latter samples was utilized.

TABLE 1. Respiration and Glycolysis by Platelets

Experiment No.	Composition of sample*	Incubation conditions	Respiration (μl O_2)	Glycolysis (increase in lactic acid in mg)	Sugar consumption (in mg)
1	A	O_2	1 345	3,9	3
	B	O_2	872	3,6	29
2	A	O_2	—	0,60	2,1
	B	O_2	—	0,85	19,2
3	A	O_2	—	3,5	3,4
	B	O_2	—	5,9	12,8
	B	N_2	—	11,1	50,6
4	A	O_2	1 080	2,5	1,3
	B	O_2	976	2,7	9,5
	B	N_2	—	6,2	26
5	A	O_2	1 832	3,2	7,0
	B	O_2	1 758	2,6	8,4
	B	N_2	—	11,4	47

*(A - without additional glucose; B - with additional glucose.

The data presented show that platelets possess rather vigorous energy metabolism.

TABLE 2. Effect of Various Concentrations of Glucose on Respiration and Glycolysis by Platelets

Respiration and glycolysis	Composition of samples			
	no glucose added	glucose added		
		200 mg%	600 mg%	4,000 mg%
O_2 consumption in μl per hour	1,836	1,532	1,668	1,338
Glycolysis (increase in lactic acid per mg in 2 hr).	6.4	7.2	6.0	9.6

TABLE 3. Respiration and Glycolysis by Platelets During Storage (series no. 8)

Indices	Days of storage					
	first		fifth		tenth	
	no glucose added	glucose added	no glucose added	glucose added	no glucose added	glucose added
O_2 consumption, in μl	704	564	566	600	352	330
Content, in mg, of:						
lactic acid	9.0	9.0	20	21.6	23.8	28.2
sugar.	42	381	22	381	18	359
ATP	0.30	0.36	0.40	0.42	0.29	0.22
inorganic P.	1.80	1.54	1.87	1.71	1.75	1.70
Retraction, in % . .	77	81	45	63	–	–

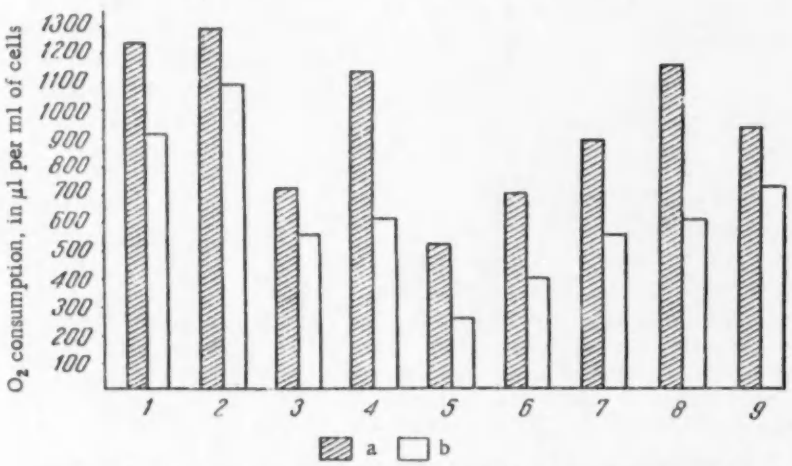

Figure. Reduction in 0_2 consumption by a platelet suspension following addition of glucose. a – Samples without additional glucose; b – Samples with additional glucose.

Subsequent experiments were for the purpose of determining whether it is possible to keep platelets in a viable condition. To this end, our thrombocyte suspension was divided up into individual samples, which were stored in the refrigerator at +4° – +6° for ten days, and examined on the first, fifth, and tenth day of storage. During storage, the analyses mentioned earlier were carried out, and in addition, clot-retraction capacity was determined as an indication of the viability of these formed elements. The amount of retraction was noted after a one-hour incubation of plasma together with platelet suspension diluted with a solution of calcium chloride. As the retraction capacity is reduced, the density of the clot formed decreases, more fluid being

retained in it. The amount of fluid remaining was determined in the graduated portion of a special flask in which the determination was made and was compared with the initial value, which was taken as 100%.

Study of the platelet suspension during the storage process showed that the respiration rate gradually falls, but remains at a sufficiently high level during the storage intervals investigated. The increase in lactic acid sometimes stops in the first few days of storage, or continues until the end of the observation period. The inorganic phosphate content does not show any substantial changes. The quantity of ATP (or rather, of readily hydrolyzable P) is reduced somewhat, but this fraction does not disappear entirely (Table 3).

The results are given on the basis of 1 ml of densely packed platelets. Sometimes an increase in the content of readily hydrolyzable P was observed during the final periods of storage, as in the case of storage of erythrocytes. It should be noted that the stability of this phosphorus fraction in platelets can be regarded as one of the characteristic features of these formed elements. The retraction capacity of the platelets is largely preserved for several days, being more pronounced in samples to which glucose was added. Maintenance of energy metabolism at a rather high level for a prolonged time argues in favor of retention of the physiological capacities of the platelets.

Our data do not conform to the generally accepted view that platelets are extremely unstable. The preservation of respiration for a long period, as well as the relative constancy of the content of readily hydrolyzable phosphate, does not support this view.

On the basis of the observed stability of platelet metabolism in storage, we may conclude that preservation of these elements largely depends on storage conditions. The duration of platelet storage in a physiologically normal condition can apparently be increased by the selection of factors that will promote the preservation of their metabolism.

SUMMARY

Platelets isolated from the blood of donors vigorously consume oxygen and carry on glycolysis, under both anaerobic and aerobic conditions. Addition of glucose brings about a reduction in the oxygen consumption rate. During storage of a platelet suspension under sterile conditions at 4° C., respiration and the ability to accumulate lactic acid are preserved. The level of the readily hydrolyzable phosphorus fraction remains relatively constant.

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EFFECT OF DEEP HYPOTHERMIA ON CARBOHYDRATE AND PHOSPHORUS METABOLISM IN BRAIN TISSUE

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In a number of investigations [6, etc.] it has been shown that when the body temperature of warm-blooded animals is artificially lowered, the rate of oxidative processes, both in the whole organism and in the tissues of the brain, is distinctly reduced. Despite this fact, the concentration of such energy-yielding substances as adenosinetriphosphate (ATP) remains at the normal level; the balance of oxidative and glycolytic processes also is not disturbed [1–4, etc.]. In lowering the body temperature of animals, the authors mentioned did not, as a rule, study the effect of temperatures below 20°, since the animals quickly died from cardiac or respiratory arrest when body temperature was further reduced.

Our purpose in the present study was to ascertain whether disturbances occur in the balance of oxidative and glycolytic processes and in the maintenance of a high level of energy-yielding substances, when the level of body temperature is reduced still further than in the works mentioned above. Such studies are of interest in view of the fact that a special method of warming has been described which permits animals to recover even after their body temperature has been reduced almost to 0°, with cessation of respiration and cardiac activity [5].

METHOD

Cats were used as experimental animals. Efforts to carry out cooling of animals as described by O. N. Savchenko [4] in experiments on rabbits (injection of 70 mg/kg of amobarbital sodium, and placing in ice water) were unsuccessful, since respiration and cardiac activity stopped when the body temperature was lowered to approximately 20°.

The following method of achieving deep hypothermia in cats was more successful. The cat was placed in a 16-liter glass vessel, surrounded by ice. A piece of cotton moistened with ether was placed on the cat's nose. As soon as the animal fell asleep, the ether-filled cotton was removed and the vessel was covered with a lid. Carbon dioxide exhaled by the cat into the closed vessel exerted a narcotic effect, cutting off the activity of the temperature-regulating centers in the first period of hypothermia. During the cooling process, the animal was taken out from time to time for measurement of temperature, and was also watched for respiration and cardiac activity (as indicated by the pulse). During the first hour, the body temperature fell 4°, on the average. After three or four hours, more rapid cooling was begun, by surrounding the cat with ice-filled bags in an open vessel. It was thus possible to reduce the body temperature to 14° while maintaining respiration and heartbeat.

When cooling was complete (five to six hours after the beginning of the experiment), the cat's head was frozen by immersion in liquid oxygen; the animal was decapitated, and the brain was removed and ground in a mortar. The brain proteins were precipitated with trichloroacetic acid (2 ml of 10% CCl_3COOH and 2 ml of water were added to 1 gm of tissue), and the protein-free filtrate was analyzed for inorganic phosphorus (by the method of Fiske and Subbarow), ATP labile phosphate (as readily hydrolyzable phosphorus in a mercury precipitate), phosphocreatine (Alekseeva's method), and lactic acid (method of Friedemann, Cotonio, and Shaffer), with precipitation of proteins with zinc hydroxide (Vladimirov and Urinson's method) and precipitation of carbohydrates with a copper sulfate-calcium hydroxide mixture.

RESULTS

To have the necessary data for a comparison under the conditions used for freezing the brain, we performed the first group of experiments on animals with normal body temperature (Table 1).

The results obtained are marked by a small scatter around the mean, which enables us to conclude that the methods used for freezing the brain and preparing brain tissue for analysis are satisfactory. The results of experiments on animals with reduced temperature are presented in Table 2.

Comparison of the mean values in Tables 1 and 2 shows that they are very close to each other. Thus, when the body temperature of the experimental animals is reduced more than 20°, the level of all the constituents investigated is the same as it is if the temperature is normal.

TABLE 1. Content of Inorganic Phosphorus, ATP Labile Phosphorus, Phosphocreatine Phosphorus, and Lactic Acid in Brain Tissue of Cats with Normal Temperature

No.	Phosphorus (mg %)			Lactic acid (mg %)
	inorganic	ATP	phospho-creatine	
1	16	16,2	8	30
2	11	12,5	9	32
3	13	11,7	7	29
4	14	12,4	7	33
5	13,7	12,5	7,6	29
Mean	13 5	13	7,7	31

In several instances, in spite of the precautionary measures taken, cardiac arrest occurred. When this fact was discovered, the animal was immediately frozen. The period of time between the moment of cardiac arrest and freezing apparently did not exceed 2 - 4 minutes; nevertheless, marked changes were observed in all the investigated components in such cases, particularly in the concentrations of phosphocreatine and lactic acid (Table 3).

TABLE 2. Content of Inorganic Phosphorus, ATP Labile Phosphorus, Phosphocreatine Phosphorus, and Lactic Acid in Brain Tissue of Cats with Reduced Temperature

No.	Body temperature	Phosphorus (mg %)			Lactic acid (mg %)
		inorganic	ATP	phospho-creatine	
1	24°	25	10,6	4,8	27
2	21°	13	11	6,8	28
3	16°	16	11,5	9	—
4	14°	12,2	12,7	6,6	27,3
5	14°	12	11,4	7	24
6	15°	12,5	11,7	7,5	26
Mean	—	15,1	11,5	7	26,4

In the literature one encounters the assumption that hypothermia is accompanied by brain hypoxia, even when cardiac activity and respiration are maintained. O. N. Savchenko [4] showed that there is no hypoxia of the brain in rabbits when the body temperature is lowered to 22 - 20°. In our experiments on cats at lower temperatures, in every case in which respiration and cardiac activity were maintained, no signs of hypoxia were observed; the level of such energy-yielding substances as ATP and phosphocreatine was not reduced, and breakdown of phosphorus compounds and accumulation of lactic acid did not occur. But as soon as the heartbeat stopped, all the measured indices of carbohydrate and phosphorus metabolism showed a markedly hypoxic condition during the first two to four minutes: The phosphocreatine concentration fell to zero in every case, without exception; the ATP concentration dropped 50%; and the levels of inorganic phosphorus and lactic acid doubled.

In spite of the low body temperature and the slowing of biochemical processes under these conditions, these changes occurred with extreme rapidity - within two to four minutes. This gives us a better understanding of the results of the investigation of Andjus and Smith [5], who were able to maintain life in rats whose body temperature was reduced almost to zero, with cardiac arrest. Recovery was possible only in those cases in which warming was begun in the region of the heart, by means of ultrahigh frequency currents.

The supplying of oxygen and glucose to the brain, begun while the body temperature is still very low, plays a decisive role in restoring the normal balance of oxidative and glycolytic processes and the requisite reserve of energy-yielding substances, and thereby, in restoring brain activity.

TABLE 3. Content of Inorganic Phosphorus, ATP Labile Phosphorus, Phosphocreatine Phosphorus, and Lactic Acid in Brain Tissue of Cats in Hypothermia With Cardiac Arrest

No.	Body temperature	Phosphorus (mg %)			Lactic acid (mg %)
		inorganic	ATP	phospho-creatine	
1	20	32	5,6	0	58
2	15	30	5	0	60
3	13	22,5	7,1	0	55

SUMMARY

When the body temperature of cats is reduced to as low as 14° C, the concentrations of inorganic phosphorus, ATP, phosphocreatine, and lactic acid in the brain tissue remain unchanged. This points to an absence of hypoxia or any other condition that might disturb the balance between glycolytic and oxidative processes. Arrest of the cerebral blood supply, even for a short time, under hypothermia results in breakdown of energy-yielding substances; this should be taken into consideration when the animal is to be brought back to the normal temperature level.

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THE EFFECT OF HYPOXIA AND ACTH ON THE CARBOHYDRATE RESERVE IN TISSUES OF THE WHITE RAT

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must have been "as" in the original. Looks as if it had been changed to "is" on proof - which makes it senseless.

A number of investigations [1, 2] have shown that when an organism's oxygen supply becomes inadequate, the role of the carbohydrates is the most important, and for some organs the only source of energy increases markedly. Changes that occur in carbohydrate metabolism under these conditions often result in disturbances of other types of metabolism, which may promote fatty infiltration of tissues, accumulation of various incompletely oxidized metabolic products, and even reduction of the potential of high-energy compounds. Metabolic disturbances accompanying oxygen insufficiency continue to remain for some time even after the influences of the reduced partial pressure of oxygen has been removed from the organism. The degree of reversibility of these disturbances largely determines the reaction of the whole organism, as well as of individual organs, to hypoxia. This problem, however, and especially the problem of the state of tissue carbohydrate reserves have been extremely inadequately studied, since the majority of investigations have been concerned with the dynamics of the carbohydrate reserve in only the blood and brain, neglecting the reserves of other tissues.

In addition, such investigations have been conducted, as a rule, in chronic experiments, in which the animals were subjected to the action of hypoxia for many hours and even for several days, and the development of adaptation under these conditions did not permit estimation of early changes in the state of the tissue carbohydrate reserve.

Studies of shifts in metabolism during hypoxia are intimately related to the search for agents and methods capable of stopping these shifts and elevating the organism's resistance to oxygen hunger. Data have recently appeared [4] which indicate that preliminary administration of adrenocorticotropic hormone from the anterior lobe of the pituitary (ACTH) sharply increases the percent survival of animals under conditions of acute oxygen hunger. The causes of this phenomenon are still unclear in many respects.

In the present study, we investigated the degree and persistence of shifts occurring in the carbohydrate reserve of liver, skeletal muscle, and heart of rats, as well as the effect of preliminary injection of ACTH on the dynamics of the carbohydrate reserve in these tissues, under conditions of acute oxygen hunger and afterward.

METHOD

The work was done in a 64-liter pressure chamber on sexually mature male rats weighing 220-250 gm. The animals were subjected to experimentation one at a time, on an empty stomach. The pressure chamber was equipped with inflow - outflow ventilation and a mercury manometer. The pressure within the chamber was held at a level of 180 mm Hg, corresponding to a "height" of 10,500 meters, for an hour. "Ascent" and "descent" were brought about at a rate of 2100 meters per minute. The animals were killed by decapitation under light ether anaesthesia. Tissues to be analyzed were removed as quickly as possible, fixed in liquid oxygen, and ground in a mortar, after which appropriate portions were taken. Glycogen content was determined by means of a color reaction with concentrated sulfuric acid [5], and lactic acid content by means of a color reaction with p-hydroxydiphenyl [6]. In studies of the recovery period (posthypoxic period), after one hour in the pressure chamber the rats were transferred to an environment in which the partial pressure of oxygen was normal. Finally, in a third series of experiments the animals were first injected subcutaneously with two units of ACTH per day for six days. On the seventh day, the animals were subjected to experimentation. The total dose of hormone was 12 units per rat.

RESULTS

In view of the fact that there were sizable individual variations in glycogen and lactic acid content in the tissues, all the numerical material was treated statistically. The mean values obtained are summarized in the table.

Distinctive changes are observed in the state of the tissue carbohydrate reserve in rats preliminarily injected with ACTH. As may be seen from the data in the

Glycogen and Lactic Acid Concentrations in Rat Tissues Under Various Physiological Conditions

Group No.	Experimental conditions	Liver				Skeletal muscle				Heart			
		number of determinations	amount of glycogen (mg %)	number of determinations	amount of lactic acid (mg %)	number of determinations	amount of glycogen (mg %)	number of determinations	amount of lactic acid (mg %)	number of determinations	amount of glycogen (mg %)	number of determinations	amount of lactic acid (mg %)
1	Control	24	4213±252	21	33±2	24	701±21	22	48±3	22	440±15	21	110±4
2	Oxygen hunger ("height" of 10 500 meters; time 60 minutes)	14	987±106	13	90±6	14	540±27	15	124±7	15	426±17	15	160±6
3	Posthypoxic period	15	2301±311	15	25±1	15	590±39	15	40±3	15	456±19	14	114±6
4	Injection of ACTH, normal conditions	10	4240±321	10	36±2	10	745±19	10	44±4	10	429±12	10	96±6
5	Injection of ACTH, oxygen hunger	10	2381±353	10	40±2	10	621±37	10	55±5	10	425±34	10	120±7
6	Injection of ACTH, post-hypoxic period	5	3478±205	5	38±3	5	753±21	5	40±3	5	443±22	5	91±5

Note: Each figure is the mean of values obtained in two parallel experiments.

table, under normal conditions the effect of the hormone on the state of the tissue carbohydrate reserve was almost imperceptible. We observe only a slight increase in the glycogen of skeletal muscle of experimental rats in comparison with the controls. The sensitivity of the animals to injection of hormone under normal conditions is apparently small; in every instance it has no noticeable effect on the tissue carbohydrate reserve. There is only a

slight degree of elevation of lactic acid utilization in skeletal muscles and myocardium, where a tendency toward reduction of lactic acid content is observed. A different picture is seen in oxygen hunger, where preliminary injection of ACTH results in a considerably smaller loss of glycogen by the liver and skeletal muscle, in comparson with animals that did not receive the hormone. But accumulation of lactic acid in this case is markedly reduced. One has the impression that the hormone promotes the preservation of tissue carbohydrate reserves. It is possible that under conditions of oxygen hunger gluconeogenesis from proteins and fats is enhanced in the tissues under the influence of ACTH, and that an inhibitory effect of ACTH on the hexokinase reaction also appears in these circumstances. As a result, the glycogen reserve in liver and skeletal muscle increases. It should be noted, however, that the hormonal effect influences the level of the glycogen reserve in only liver and skeletal muscle, and has no effect on its level in the heart, where only the reduced lactic acid accumulation is seen.

The normalizing effect of the hormone can also be noted in the posthypoxic period, when a considerably greater degree of restoration of the glycogen reserve in the liver and skeletal muscle is clearly displayed. At the same time, the content of lactic acid in liver, skeletal muscle, and heart returns to normal. Thus, preliminary injection of ACTH has a favorable effect on the state of the tissue carbohydrate reserve, reducing depletion of it in hypoxia and hastening restoration of it in the posthypoxic period. In both cases it causes increased utilization of lactic acid by the tissues. Apparently, the typical adaptive effect of this hormone on metabolism is manifested in this case.

It is possible that the increased percent survival of rats preliminarily given ACTH is connected with the normalizing effect of ACTH on tissue carbohydrate reserves, which porbably causes more complete mobilization of tissue compensatory mechanisms that increase the organism's resistance to oxygen hunger.

SUMMARY

The author studied the effect of preliminary ACTH administration of glycogen and lactic acid concentrations in liver, skeletal muscle, and myocardium of male white rats during oxygen deficiency resulting from reduced barometric pressure and in the posthypoxic period. It was shown that preliminary administration of ACTH (with a total dose of 12 units) to rats later subjected to acute oxygen deficiency markedly reduces the fall in glycogen content in liver and skeletal muscle and simultaneously inhibits the accumulation of lactic acid in these organs. It also leads to more rapid restoration of liver and skeletal muscle glycogen reserves, and more complete elimination of excess lactic acid, during the posthypoxic period. Under conditions of hypoxia and posthypoxia, preliminary administration of ACTH has no significant effect on the cardiac glycogen reserve.

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THE EFFECT OF GANGLION-BLOCKING DRUGS ON THE CONDUCTION VELOCITY OF NERVOUS EXCITATION IN THE SYMPATHETIC GANGLIA

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As we know one of the fundamental characteristics of the functions of the nervous system is the velocity with which a stimulus is propagated. This factor is especially significant when applied to the interneuronal transmission of nerve impulses. In addition, as the work of V. V. Zakusov and co-workers has shown [2, 3, 5, 6, 7, 8], the amount of synaptic suppression is one of the more precise criteria for the evaluation of substances which act on the central nervous system.

Starting with this information, and continuing our attempts to elucidate the mechanism of action of ganglion-blocking substances [9, 10, 12], we undertook the present work, which is a study of the influence of ganglion-blocking agents on the conduction velocity of nervous excitation in the sympathetic ganglia.

METHOD

The experiments were performed on anaesthetized cats. The anesthetic used was either urethane (1 to 1.5 mg/kg by intraperitoneal injection) or a mixture of chloralose (80 mg/kg) with urethane (0.5 g for each animal, injected intravenously). The bioelectric potentials of the postganglionic fibers of the superior cervical and stellate ganglia were discharged through bipolar platinum electrodes. The preganglionic fibers were stimulated by successions of single squarewave supramaximal stimuli (3 to 5 per series) with an interval of 5 to 60 seconds between each stimulus, the duration of the latter being 0.1 to 0.5 milliseconds. The latent period was measured between the artifact of the stimulus and the commencement of the potential (Figs. 2a and b).

In experiments on the stellate ganglion the bioelectric potentials were discharged from the inferior cardiac nerve. All the other postganglionic fibers, as well as the 1-5 rami communicantes, were cut. The sympathetic thoracic chain was either tied or cut on a level with the 5th or 6th ganglion. Thus the stellate ganglion was completely isolated from any influence whatever on the central nervous system. The blood supply was meanwhile maintained. The stimulating electrodes were placed on the thoracic sympathetic chain, usually between the 3rd and 4th, or 4th and 5th ganglia. Artificial respiration was used in the experiments.

We recorded the potentials of the postganglionic fibers of the superior cervical ganglion when the peripheral segment of the cervical sympathetic trunk (presynaptic fibers) was submitted to electrical stimulus.

Among the ganglion-blocking substances tested were tetraethylammonia (0.5 to 5 mg/kg), hexonium (0.5 to 7 mg/kg), pentamine (0.5 to 10 mg/kg), and mecamine (0.5 to 6 mg/kg). In addition, for purposes of comparison, experiments were performed with novocain (15 to 25 mg/kg) and barbamyl (10 to 20 mg/kg). The substances were injected into the femoral vein.

RESULTS

The results obtained indicate that all the ganglion-blocking substances which we tested decrease the conduction velocity of sympathetic ganglia excitation. This applies equally to the superior cervical and the stellate ganglia. Any alteration in the latent period, consequent on a considerable reduction in the amplitude of the bioelectric potentials, was usually expressed in the form of an increase of 0.5 to 2 milliseconds.

It must be stated that, in all the experiments, only supramaximal stimulation was applied. Comparison of results was thereby noticeably simplified, quite apart from the fact that supramaximal stimulation provided the most reliable conditions. It did not appear to be worthwhile to apply different amplitudes of stimulation in the presence of the substances under test, as an increase in the strength of stimulus from a liminal to a supramaximal value was not accompanied by any noticeable change in the latent period. Evidently the initial part of the biological currents (before which the latent period is measured) is the result of the activity of neurones with the least excitability discharging with liminal stimulation of the preganglionic fibers.

Changes in the latent period induced by ganglion-blocking agents may be considerably greater than those

mentioned earlier. Thus, as shown in Fig. 1, the conduction time of the stimulus in the superior cervical ganglion is increased by 4 milliseconds under the influence of tetraethylammonia (1 mg/kg). A marked decrease in the amplitude of the biological currents was observed simultaneously.

Analogous results are shown in Table 2. Following injection of hexonium 5 mg/kg in divided doses spread over 25 minutes (1+1+1+2 mg/kg) the conduction time in the stellate ganglion increased by 3.7 milliseconds. At the same time the potential noticeably decreased.

However, an increase in the latent period takes place only up to a certain limit. Thus, in our investigations into ganglion-blocking drugs, the latent period did not extend beyond 5 milliseconds. A further increase in the dose led to complete block of ganglionic transmission.

In no experiment was there observed a reduction in the latent period, even with small doses.

Having established the possibility of ganglion-blocking agents having a retarding effect on the conduction velocity of excitation in the sympathetic ganglia, we thought that it would be interesting to study other substances which showed marked ganglion-blocking properties. We therefore investigated novocain and barbamyl. These substances were selected because experiments had

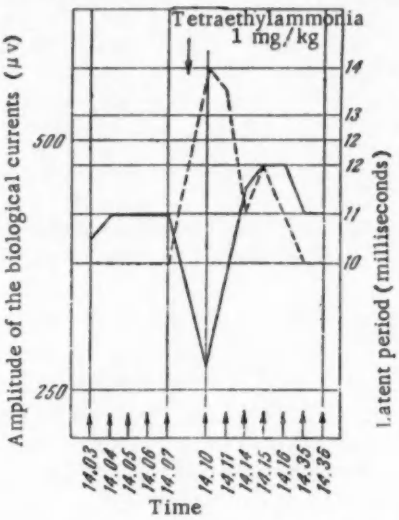

Fig. 1. Influence of tetraethylammonia on conduction velocity of excitation in the superior cervical ganglion. Continuous line—amplitude of postganglion potentials; broken line—latent period (period between the artefact of excitation and onset of potential; vertical arrows ( ) above the time scale—single supramaximal stimuli.

shown that they definitely depress the transmission of nerve impulses in the sympathetic ganglia [1, 9, 11, 15]. It was established that both compounds have a depressive effect on the velocity of propagation of stimuli. Barbamyl (15 mg/kg) and novocain (25 mg/kg) noticeably increased the transmission time (Fig. 3). This effect, in the case of both substances, was always accompanied by a decrease in the amplitude of the biological currents. Pessimol* retardation developed noticeably earlier than in the control.

It follows from the data shown that ganglion-blocking substances lower the synaptic velocity in the sympathetic ganglia. The latent period is usually increased by 1 to 3 milliseconds, which is quite considerable if one takes into account that the true synaptic suppression for the group of cells with the lowest liminal excitability is

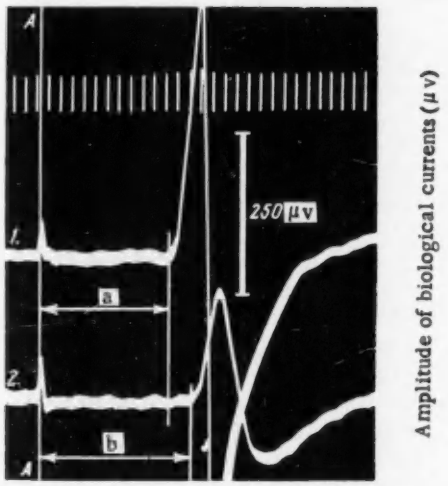

Fig. 2. Biological currents of postganglionic fibers (stellate ganglion). 1) Before; 2) after injection of hexonium 5 mg/kg (divided doses over a period of 25 minutes 1 + 1 + 1 + 2 mg/kg); line A - A, corresponding to the time of stimulation of the preganglionic fibers; a, b—values of the latent period before and after administration of hexonium; vertical lines in the upper part of the figure—time (one division = 2 milliseconds).

2 to 3 milliseconds [13, 14, 16]. An increase in the latent period is evidently connected with the fact that the ganglion-blocking substances extend the time of development of synaptic potential up to a critical value related to the discharge of the ganglionic cells. Consequently we may suppose that the transition of the local stimulus into a diffuse stimulus becomes more difficult. This latter effect must obviously be considered one of the fundamental manifestations of the action of ganglion-blocking substances.

Changes in the velocity of interneuronal transmission, observed when ganglion-blocking substances were applied, are usually accompanied by a noticeable decrease in the amplitude of the biological currents of the postganglionic fibers. In addition, as shown earlier [9, 10, 12], the substances which we tested lower the functional mobility (lability) of the superior cervical ganglion, which can be judged by the displacement of the pessimal reaction towards the lower frequencies. Evidently, in-

* Transliteration of Russian - Publisher's note.

crease in synaptic retardation also testifies to lowering of lability, the fundamental character of which is the

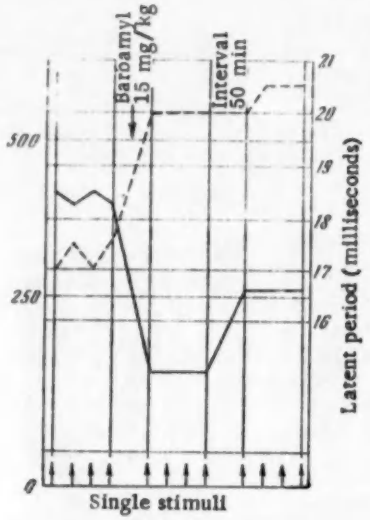

Fig. 3. Influence of barbamyl on conduction velocity of excitation in the stellate ganglion. Continuous line amplitude of bioligical currents of postganglionic fibres; broken line latent period, intervals between stimuli of each series (of 4 stimuli) 5 seconds; the time of stimulus is indicated by the vertical arrows ( ).

"velocity of those elementary reactions which accompany functional activity" (N. E. Vvedenskii [4]).

In addition to ganglion-blocking agents, barbamyl and novocain were investigated. This part of the work was done to show whether a change in the value of the latent period is also produced as a general rule by other pharmacological groups which have the ability to depress ganglionic transmission.

We established in our experiments that barbamyl and novocain, in addition to the ganglion-blocking agents, increase the latent period, at the same time noticeably decreasing the lability of the ganglionic neurons and reducing the amplitude of the postganglionic potentials.

Thus, from the facts discovered, we can conclude that a decrease in the conduction velocity of ganglionic excitation may be regarded as a general manifestation of the action of substances which possess gangliotic properties, regardless of the mechanism of their depressive influence.

SUMMARY

Experiments were performed on cats under urethane-chloralose anaesthesia. The author studied the effect of ganglioblocking drugs, novocain and barbamyl on the condition velocity (latency) of excitation in the superior cervical and stellate ganglia. Discharges from postganglionic fibers were recorded in response to a single supramaximal electric stimulation of the preganglionic trunk.

All the preparations investigated viz., tetraethylammonium (0.5 - 10 mg/kg), hexonium (0.5-7 mg/kg) pentamine (0.5-10 mg/kg), mecamine (0.5-6 mg/kg), novocain (15-20 mg/kg) and barbamyl (10-20 mg/kg) provoked a lengthening of the interval between the artifact of the exciting stimulus and the response discharge by 0.5-4 milliseconds, which was parallelled by a decrease of the discharge amplitude. Reduction of the synaptic conduction velocity in the ganglia occuring under the effect of the substances under investigation is regarded as a consequence of decreased functional mobility of the ganglionic neurons; it is viewed as a general manifestation of the action of gangliotics, irrespective of the mechanism of their depressive effect.

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CHANGES IN THE OPTIC CHRONAXIE AND THE ELECTROENCEPHALOGRAM DUE TO DRUGS ACTING ON THE TRIGEMINAL NERVE ENDINGS

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The reactions of the nervous system to an increasing intensity of excitation arising in response to sub- and superthreshold stimuli enable the general pattern of reflex activity to be established [1, 4]. In the study of this problem great importance is attached to comparison of the dynamics of the central processes in response to the action of stimuli on different peripheral analyzers, as well as to the quantitative evaluation of the level of excitation during stimulation of the same receptors.

Chemical substances present in the surrounding air are known to act on the different receptors of the upper respiratory tract. Some substances show a well-marked action on the receptors of the olfactory nerve, whereas others act mainly on the trigeminal nerve endings and, in contrast to the first which give rise to sensations of smell, they cause irritation as, for example, a tickling of the back of the throat, cough, and other effects.

In the present research we studied the reflex effect of a sulfuric acid aerosol, which is distinguished by a marked irritating action in the innervation zone of the trigeminal nerve.

As indices of the effect of this substance we used the EEG and the optic chronaxie. The sulfuric acid aerosol is one of the group of existing hazards. The determination of the threshold of reflex action of this substance by the changes in the EEG and optic chronaxie can, therefore, serve as the basis for the limiting permissible concentration of sulfuric acid in the atmospheric air in centers of population.

EXPERIMENTAL METHOD

In order to give a predetermined dose of the sulfuric acid aerosol, the method recommended by the Commission on the Limiting Permissible Concentrations of Atmospheric Pollutants [2] was used. At a short distance in front of the test subject's face, in a soundproof conditioned-reflex chamber, was suspended a cylinder through which air was circulated at the rate of 30 liters/min, passing through a system of filters which retained odoriferous substances present in the air in the building. The air was delivered by means of a rotating air pump. Air entered the cylinder, through which the subject breathed, continuously throughout the period of the investigation. At a given moment the addition of a definite concentration of sulfuric acid aerosol to the pure air was made by means of a system of cocks from the supply vessel.

The optic chronaxie was investigated six times at intervals of 3 min on a model GIF condenser chronaximeter, and between the third and fourth determination the subject inhaled air with the addition of a certain quantity of sulfuric acid aerosol for 2 min. As a rule the rheobase remained unchanged during the investigation.

The electrical activity of the brain was investigated by means of a 4-UN44-channel amplifier with a ChR-1 ink-recording attachment from the experimental factory of the AMN SSSR. The main response reaction which we used for evaluating the influence of the olfactory stimulus was desynchronization of the electrical activity or depression of the α-rhythm. During the time of development of the conditioned reflex, a light which caused desynchronization was used for reinforcement. The investigations were carried out on 10 healthy persons with normal olfactory function and with normal respiratory passages.

EXPERIMENTAL RESULTS

The concentrations of sulfuric acid aerosol which, according to the verbal response of the subjects tested, had an irritant action (tickling at the back of the throat) were regarded as threshold values. The minimal dose of this substance to produce the threshold effect was 0.6 mg/m^3. In less sensitive subjects, however, its value was higher - 0.85 mg/m^3.

As shown by the optic chronaxie, an effect of the sulfuric acid aerosol on the central processes was also apparent when acting in a concentration of 0.6-0.85 mg/m^3.

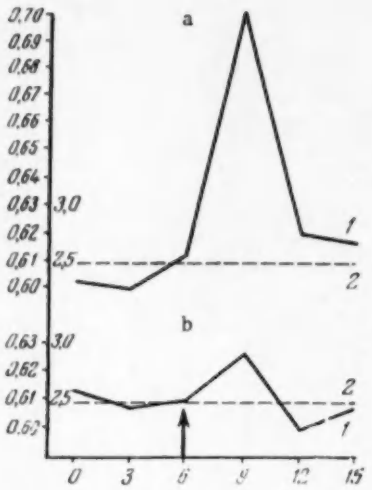

Fig. 1. Increase in the optic chronaxie following the use of sulfuric acid aerosols in threshold concentrations. On the axis of abscissas on the left is shown the chronaxie (in μf), on the right the rheobase (in v); on the axis of ordinates is shown the time (in min). The use of sulfuric acid aerosol in a concentration of 0.73-0.75 mg/m^3 is shown in (a) and in a concentration of 0.60-0.63 mg/m^3 in (b). 1 – chronaxie; 2 – rheobase. The arrow shows the moment of giving the sulfuric acid aerosol.

It can be seen in Fig. 1 that a sulfuric acid aerosol, in a concentration of 0.6-0.63 mg/m^3, causes an insignificant rise in the optic chronaxie. In their verbal response the subjects stated that, in these doses, the aerosol caused a vague sensation of tickling in the back of the throat. In higher concentrations (0.73-0.75 mg/m^3), the sulfuric acid aerosol caused a greater increase in the optic chronaxie, and in their verbal response the subjects stated that there was now an obvious tickling in the throat. When concentrations of 2.4 mg/m^3 and over were used, isolated bouts of coughing took place.

It follows from these facts that the influence of the sulfuric acid aerosol on the reflex reactions, as measured by chronaximetry, begins when the intensity of excitation which it causes is equal to that at which arises the specific reaction for this substance and its reflection in the verbal response. The influence of the sulfuric acid aerosol on the optic chronaxie showed a characteristic trend. On repetition of the investigation after an unequal interval of time in different subjects, the initial effect of an increase in the optic chronaxie disappeared. In the subject K., for instance, the initial value of the optic chronaxie was 0.60 μf. The use of a sulfuric acid aerosol in a concentration of 0.75 mg/m^3 caused an increase in the optic chronaxie to 0.71 μf. On the second investigation the degree of increase of the optic chronaxie was smaller (0.69 μf), and on the fourth it was insignificant (0.62 μf).

During the study of the electrical activity of the brain we were interested to learn whether the EEG would change during the isolated use of the sulfuric acid aerosol in subthreshold concentrations in accordance with the verbal response and the chronaximetry findings. The investigations showed that the isolated use of this substance in subthreshold concentrations did not cause desynchronization in the EEG.

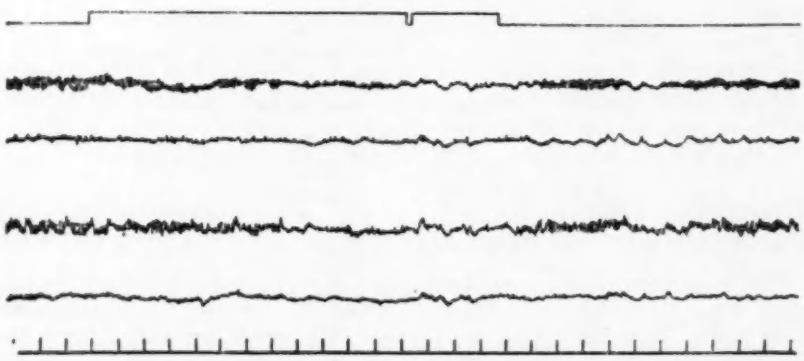

Fig. 2. Conditioned reflex desynchronization in response to the use of a sulfuric acid aerosol in a subthreshold concentration of 0.4 mg/m^3. Twenty third combinations of the action of the sulfuric acid aerosol with a continuous light. Legend (from above down): Stimulus marker, EEG of the occipital area of the right hemisphere, EEG of the temporal area of the right hemisphere, EEG of the occipital area of the left hemisphere, the same in the temporal area, time marker (1 sec).

In the cases when threshold concentrations of the sulfuric acid aerosol were used, desynchronization was observed in the EEG for 1-2 sec. The desynchronization was apparent both during the time of the isolated action of the sulfuric acid aerosol and immediately after the cessation of its supply into the air inhaled by the subject. The effect of desynchronization was unstable and after 2-3 repetitions it was completely extinguished; it was not restored even during the use of the sulfuric acid aerosol on the subsequent days of the investigation.

Changes in the electrical activity of the brain in the form of desynchronization of the EEG thus took place when the use of the sulfuric acid aerosol induced excitation of sufficient intensity to alter the course of other reflex reactions. We have in mind particularly the phosphene reaction, an index of which is the optic chronaxie.

From the results of our investigations the question arose whether subthreshold concentrations of sulfuric acid aerosol cause excitation in the central nervous system. The reply to this question was given by research in which a conditioned reflex change in the electrical activity of the brain was developed in response to subthreshold concentrations of this substance. As conditioned reflex stimulus we used a sulfuric acid aerosol in a subthreshold concentration of 0.4 mg/m^3. The action of the aerosol in isolation was continued for 15 sec, after which a continuous light was applied for 5 sec as reinforcement. A conditioned reflex was established at the 23rd combination of the sulfuric acid aerosol and the light (Fig. 2). During the use of the aerosol alone, desynchronization was observed in the EEG. If a threshold concentration was used as a stimulus, the conditioned reflex was established much more quickly. At a sulfuric acid aerosol concentration of 0.63 mg/m^3, the reflex was established after 6-8 combinations.

When the character of the electrical activity of the brain in response to the use of substances acting mainly on the trigeminal and olfactory nerve endings, respectively, in superthreshold concentrations* was compared, attention was drawn to certain distinctive features of the reaction to a sulfuric acid aerosol. When this substance, for instance, was used as a conditioned stimulus, it caused a less pronounced desynchronization than olfactory stimuli such as, for example, sulfur dioxide gas. The conditioned reflex change in the electrical activity was also less stable. It appeared after the sixth to eighth use of the sulfuric acid aerosol and was extinguished at the 10th-12th combination of the action of this substance with the light. A considerable difference was also observed during comparison of the rate of extinction of the effect on the EEG when substances acting on the olfactory and trigeminal nerve endings were used alone in superthreshold concentrations.

If the sulfuric acid aerosol was used in a concentration of 1.3 mg/m^3 or over, then in the case of development of a bout of coughing in response to such stimulation an increase in the degree of synchronization in the EEG was observed. With repeated stimulation, the act of coughing was depressed and desynchronization was observed in the electrical activity (Fig. 3a) which was completely extinguished after 5-8 applications of the aerosol. When sulfur dioxide was given in concentrations causing a bout of coughing, the degree of synchronization also increased (Fig. 3b). If this act took place against a background of desynchronization, this desynchronization gave way to synchronized electrical activity of the brain in the form of a changeover to α-rhythm (Fig. 3c). After the repeated action of sulfur dioxide, when the subject stated in his verbal response that he suppressed his bouts of coughing, marked desynchronization also appeared. Extinction of this change in the EEG, however, took place more slowly; it was completed after 15 applications of sulfur dioxide.

The investigations described above show that a sulfuric acid aerosol has in general the same effect on the optic chronaxie and the EEG as olfactory stimuli. The former differs in the lesser degree of the reflex effect and its more rapid extinction. These differences are evidently due to the unequal degree of corticalization of the central representation of the olfactory and trigeminal nerves.

Impulses arising in response to stimulation of the receptors of the olfactory nerve are known to pass via the third neurone to the hippocampal area of the cortex [3]. This area is included in the system of nerve structures in which a continuous circulation of nervous impulses takes place. The peculiarity of the construction of the central representation of the trigeminal nerve is that it has a lesser degree of corticalization. Hence, with sub- and superthreshold doses of substances stimulating the endings of the trigeminal nerve, the resulting excitation changes the dynamics of the cortical processes to a lesser degree than olfactory stimuli.

SUMMARY

Substances stimulating the trigeminal nerve endings exercise on the central processes the same effect as olfactory stimulants. As distinct from olfactory stimulants, these substances, when employed separately, change the EEG and the optic chronaxie only at threshold concentrations (according to verbal reports of the persons under investigation). The elaboration of a conditioned reflex occurs following the combined action of subliminal concentrations of the sulfuric acid aerosol and light. Desynchronization of the EEG appears in response to the isolated action of the sulfuric acid aerosol. Recording of the cerebral electrical activity in combination with the method of conditioned reflexes enables the detection of that minimal intensity of excitation (provoked by subliminal stimulation) whose level is sufficient to affect the cortical activity.

* The effect of subthreshold olfactory stimuli on the reflex activity is discussed by us in a paper in the press.

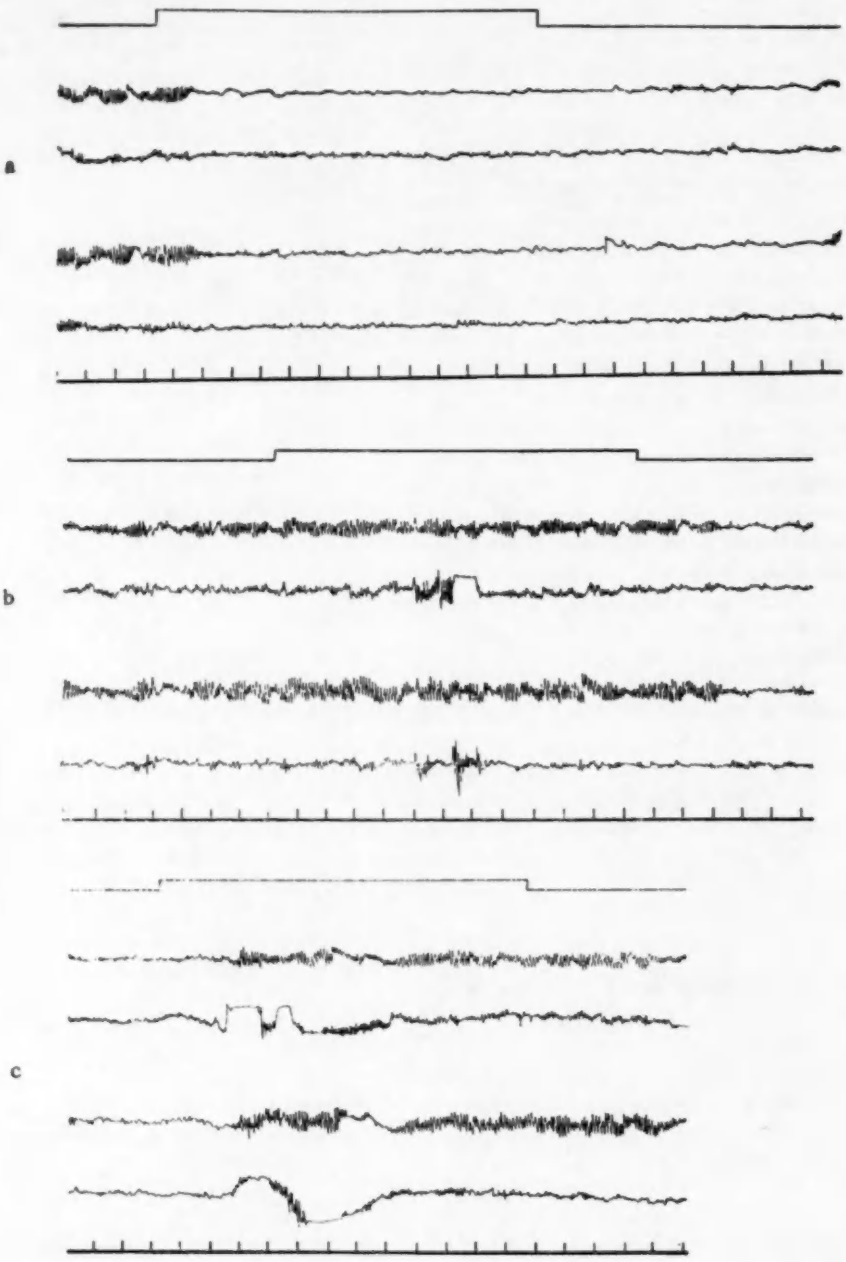

Fig. 3. The variable electroencephalographic effect in response to the isolated use of substances acting on the olfactory and trigeminal nerve endings in superthreshold concentrations. a – Desynchronization in response to the repeated use of the sulfuric acid aerosol, causing a sensation of irritation without the development of a bout of coughing (concentration of sulfuric acid 1.3 mg/m^3); b – increase in the degree of synchronization in response to the use of sulfur dioxide gas, causing a bout of coughing (concentration of sulfur dioxide 15 mg/m^3); c – replacement of desynchronization by synchronization of the electrical activity of the brain in response to the action of sulfur dioxide, causing a bout of coughing (concentration of sulfur dioxide 15 mg/m^3). Legend as in Fig. 2.

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THE INFLUENCE OF NOCICEPTIVE STIMULI ON THE CONVULSANT ACTION OF CAMPHOR

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The investigations of L. A. Orbeli [4] and many other authors convincingly showed that nociceptive stimuli, even when of short duration, cause obvious changes in the reactivity, creating a new "background" for the staging of events in the body along different lines from normal, in response to external influences.

In view of this discovery, we decided to examine the changes in the character of action of drugs when administered to animals after brief nociceptive stimulation. A series of papers from one of us [2, 3] has been devoted to the explanation of this problem; in particular, the influence of nociceptive stimuli on the convulsant action of certain analeptics has been studied.

It has previously [2] been shown that after the intraperitoneal injection of strychnine nitrate to guinea pigs in a dose of 3.0 -3.2 mg/kg, marked convulsions were observed in all the animals in control experiments. After injection of the same doses of strychnine into animals previously subjected to strong nociceptive stimulation for five minutes (stimulation of the paw with current from the mains, stepped down to 85-90 v), convulsions were either not present at all or they were very mild (only movements of the jaws or scratching were observed).

It was shown later [3] that after the subcutaneous injection of cardiazol to white rats in a dose of 70 mg/kg, clonic convulsions, sometimes becoming tetanic, were observed in control animals. When rats were injected with the same dose of cardiazol 1-10 minutes after the application of a nociceptive stimulus for ten minutes (stimulation of the paw with the current from the mains, stepped down to 30 v), although convulsions were observed in nearly all the animals, they always developed later. The number of cases of clonic convulsions turning into tetanic decreased after nociceptive stimulation.

Nociceptive stimuli thus had an undoubted effect on the intensity of the convulsant action of the analeptics studied, which had different points of main application: Strychnine acts mainly on the spinal cord and cardiazol on the midbrain.

In order to obtain certain general conclusions it was important to ascertain the effect of nociceptive stimuli on the convulsant action of camphor, the point of application of which is the cerebral cortex. The present work is devoted to the investigation of this problem.

EXPERIMENTAL METHOD

Our experiments were carried out on 200 male white mice and 4 male guinea pigs. All the mice received subcutaneous injections of camphor in a dose of 1 mg/g body weight as a 20% solution, and observations on the general condition of the animals were conducted for 2 hours and more. The time of appearance of the first signs of a convulsive state, the intensity of the fits (on a five point system: 0 - no fits, 5 - death) and their duration were recorded visually.

The experimental animals were divided into two groups: 100 mice acted as controls and 100 mice were subjected to nociceptive stimulation (compression of the root of the tail by means of an apparatus, specially constructed by V. V. Levoshin, permitting accurate dosage of the stimulus) for three minutes before the injection of the camphor. The injection of camphor was given to the mice two minutes after the end of stimulation. Each mouse was used only once in the experiment.

We carried out 8 additional experiments on 4 guinea pigs, at first conducting on each animal a control experiment, in which a 20% solution of camphor (0.2-0.24 mg/g body weight) was injected intraperitoneally, and then 10 days later, an experiment on each animal with nociceptive stimulation (the hind paw was stimulated by current from the mains, stepped down by means of a rheostat to 80 v). Stimulation was continued for ten minutes and the same dose of camphor as in the preceding experiment was given three minutes afterwards. During the experiment the animal was kept in a box specially adapted for actographic recording; tracings were made on a slowly revolving kymograph drum.

EXPERIMENTAL RESULTS

In the overwhelming majority of the mice (92 of 100), clonic convulsions, sometimes turning into tetanic, were observed in the control experiments after receiving injections of camphor in this dose. The convulsions began at different times after the injections of camphor, on the average 23 minutes. In only 29.3% of the animals (in 27 of the 92) was the latent period of the convulsions more than 30 minutes.

The mortality among the mice in the control experiments was high; more than half the total number of

animals which developed convulsions died (57 of 92). The time of death varied considerably. In the surviving animals (9 of 35) the convulsions were strong or of moderate intensity (3-4 points).

A different picture was observed in the animals subjected to nociceptive stimulation before the injection of camphor. In this group, convulsions were observed in only 68 of the 100 mice. They began only a little later than in the control animals, on the average 30 minutes after the injection of camphor. The latent period of the convulsions exceeded 30 minutes in 45.5% of the experimental animals (30 of 68).

The mortality among the animals subjected to stimulation in relation to their total number was lower (44 of 100) than in the control group, although in relation to the number of animals in which convulsions were observed, it was the same (62 and 64%). The times of death among the experimental animals varied considerably; we could detect no regularity about these variations nor in those in the control group. The intensity of the convulsions in the animals which survived after the fits was slightly less than in the control group. For instance, strong convulsions and those of moderate intensity (3-4 points) were observed in only four animals.

The experiments on mice thus showed that nociceptive stimuli decrease the frequency of development of convulsions after injection of camphor, and lengthen their latent period.

In the experiments on guinea pigs the findings were as follows: in the control experiment in guinea pig No. 1, after the injection of 0.2 mg/g of camphor convulsions began after eight minutes and lasted for 26 minutes. In the same guinea pig, after nociceptive stimulation, the beginning of convulsions was observed after 13 minutes, and they lasted 17 minutes. The intensity of the convulsive attack was the same in both experiments. In guinea pig No. 2, after injection of 0.2 mg/g of camphor, convulsions began after seven minutes and lasted ten minutes; in the same guinea pig the injection of camphor after preliminary nociceptive stimulation had no appreciable effect on either the time of onset of the convulsions (after nine minutes) or their duration (12 minutes), but the intensity of the fit was perceptibly weakened. In guinea pig No. 3, after injection of 0.24 mg/g of camphor, convulsions started after 23 minutes and lasted for 41 minutes; the injection of camphor into the same guinea pig after nociceptive stimulation produced absolutely no fit at all. Guinea pig No. 4 received an injection of 0.24 mg/g of camphor, and in the control experiment fits developed after 23 minutes and lasted for 42 minutes. In the same guinea pig the injection of camphor after preliminary nociceptive stimulation gave rise to a fit only after 91 minutes, which lasted, moreover, for 16 minutes, and was greatly weakened by comparison with that observed in the control experiment.

The experiments on guinea pigs, admittedly few in number, thus showed that nociceptive stimulation lengthened the latent period of the fit developing after injection of camphor, as a rule shortened its duration and weakened its intensity.

As the experimental results show, nociceptive stimulation after the injection of camphor has a retarding influence on the development of the convulsive state in both mice and guinea pigs. By comparing these results with those of previous experiments with strychnine and cardiazol, we can see that the nociceptive stimuli affect the action of all these analeptics in the same fashion.

Since our experiments were performed on different animals we are debarred from making a quantitative comparison of the effects obtained, although in every case, irrespective of the points of main application of the action of the drugs that were studied, nociceptive stimulation delayed the development of the convulsive attack. We are inclined to explain the similarity that we observed in the effects by the fact that, in all the investigations, under the influence of the nociceptive stimulation the development of inhibition took place in the central nervous system, and this prevented the manifestation of the convulsant action of strychnine, cardiazol and camphor. The inhibition developing in the central nervous system under the influence of nociceptive stimulation is evidently parabiotic in nature, as we have previously shown [1, 2].

In a paper recently published by V. S. Sheveleva [5], convincing results are described showing the development of inhibition in different divisions of the central nervous system under the influence of nociceptive stimulation, and proving its parabiotic nature.

SUMMARY

The effect of nociceptive (pain) stimulation on the convulsant effect of camphor was studied on white mice and guinea pigs. Camphor was injected subcutaneously in a dose of 1 mg/g to mice and intraperitoneally in a dose of 0.20-0.24 mg/g to guinea pigs. Convulsions in mice were recorded visually and evaluated according to the 5-point system (0 - absence of convulsions, 5 - death); in guinea pigs convulsions were registered actographically and recorded on a kymograph. Camphor was injected 2 minutes following discontinuance of the stimulation, which lasted 3 minutes in mice and ten minutes in guinea pigs. Experiments proved that following nociceptive stimulation, the times of onset of convulsions, as compared with controls, are postponed, the convulsions occur in a smaller number of animals, while the intensity of the already developed convulsions becomes lower. The authors hold that these effects of nociceptive stimulations on the convulsant effect of camphor are evidently caused by the development of inhibition in the central nervous system, preventing the convulsive attack.

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THE REPRODUCTION OF PARADOXICAL SENSITIVITY TO STAPHYLOCOCCAL AND CLOSTRIDIUM WELCHII TOXINS

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The phenomenon of paradoxical sensitivity to toxins, known under the name of Behring's phenomenon, has been produced by many toxins (diphtheria, tetanus, type A and B botulinus and dysentery) tested by various research workers [1- 4]. It has become accepted that summation of the toxic stimulus is possible by the injection of any exotoxin.

In order to confirm the correctness of this view, we made an attempt to reproduce Behring's phenomenon by means of daily injections of sublethal doses of staphylococcal and Clostridium welchii toxins. This parallel study was carried out because the chemical, biological and even the immunological properties of these toxins are very similar.

METHOD AND RESULTS

Experiments were conducted on mice. The toxins were injected daily, intravenously, at intervals of 24 hr. The results of the experiments with different sessional doses of staphylococcal toxin are shown in the table.

It may be seen from the figures given that not one of the 20 mice died after the repeated daily injection of 1/10 MLD. of toxin. After the injection of 1/5 and 1/3 MLD., 1 and 3 mice, respectively, died. The absence of the characteristic signs of staphylococcal toxemia, with the slow onset of death, did not allow death from other causes unconnected with the action of the toxin to be confirmed or excluded. It must be emphasized that in some experiments with a large number of injections of toxin, not one mouse died.

Similar experiments were carried out with Cl. welchii toxin. In two series of experiments, 15 mice were given daily injections of 1/5 MLD of toxin for 12 days. Not one mouse died, although each animal received more than 2 MLD.

We were thus unable to reproduce Behring's phenomenon with the two toxins tested, even in those cases when large doses of the toxins were given. The death of four mice, even if connected with the action of the toxin at all, must be explained by cumulation.

It may be concluded that, so far as their ability to cause the phenomenon of summation of toxic stimuli is concerned, staphylococcal and Cl. welchii toxins differ from those previously studied, after injection of which the phenomenon of paradoxical sensitivity was comparatively easily reproducible.

The inability of the tested toxins to cause disease (lethal poisoning) when injected in fractional doses could be attributed to two reasons.

The absence of deaths among the mice could be the result of a rapidly developing immunity. This explanation seemed to be most likely in the case of the staphylococcal toxin. The blood of laboratory animals very often contains natural antitoxin, which is evidence that these animals in the past have been exposed to the action of a similar antigen, and that their immunological structure has been modified. In these conditions, the frequent injection of fractional doses of toxin may accelerate the formation of immunity, which will develop more quickly than a lethal toxemia will develop. This hypothesis was tested in a small number of experiments. In one of these, ten mice received injections of 1/5 MLD of staphylococcal toxin. After five such injections, all the animals received 1 MLD of the same toxin. Five mice died and the remaining five survived, i.e. immunity developed in half the mice. In another experiment with the same dose

The Results of Experiments With Staphylococcal Toxin

Experiment No.	Sessional dose (MLD)	Number of series of experiments	Total number of animals	Number of injections of toxin	Result of experiment	
					no. of animals dying	no of animals surviving
1	1/3	2	22	18	3	19
2	1/5	2	20	18	1	19
3	1/10	2	20	18	—	20

of toxin (1/5 MLD) the number of injections was increased to 16, counting on a more intensive immunogenesis. When the immunity was tested, however, a dose of 2 MLD caused death of all the animals. In the same experiment five mice received 24 injections of 1/5 MLD of toxin, and then were given a dose of 2 MLD. Two of these mice died and the remaining three survived.

An analogous experiment was performed on 10 mice, which received daily injections of 1/10 MLD of staphylococcal toxin. After 17 injections, the immunity of four mice was tested by the injection of 1.5 MLD of toxin. Only one was found to be immune. The remaining six mice were tested with toxin (1.5 MLD) after 23 injections. In this group, four mice survived and two died.

In subsequent experiments it was found that immunity was regularly reproduced in the mice only after 16-24 daily injections of 1/8 MLD of toxin.

Our findings thus gave no grounds for explaining the impossibility of reproduction of Behring's phenomenon with staphylococcal toxin other than by the development of immunity. It was obvious that the disease did not develop, although the animals showed no immunity to the toxin.

The second reason for the impossibility of reproduction of Behring's phenomenon in response to injection of staphylococcal and Cl. welchii toxins could be the following. Behring's phenomenon is evidently a manifestation of the principle of summation of stimuli (in this case, toxic). If it is assumed that each injection of toxin causes a transient excitation (for less than 24 hr), summation of stimuli obviously cannot take place.

We tested this hypotesis experimentally. Experiments were carried out with staphylococcal toxin. Two groups of animals, with ten mice in each group, received injections of 1/5 and 1/10 MLD of toxin at hourly intervals. In the first case five injections were given and in the second, ten. None of the mice died, either during the experiment or the subsequent period of observation lasting a few days. Consequently, the shortening of the intervals between injections, taking account of the transient nature of the stimulation with staphylococcal toxin, had no effect.

At the same time it was found that no cumulation of the action of the staphylococcal toxin took place; the injection of 1 MLD in the course of 5 hr did not cause death of the animals.

We thus were unable to explain the conditions favoring the summation of toxic stimuli and the second hypothesis could not, consequently, be regarded as proved.

In order to discover the causes of the difference between staphylococcal and Cl. welchii toxins on the one hand and many tested bacterial toxins on the other, it is evidently necessary to pay attention to the properties which distinguish them most from other exotoxins. The hypothesis that all toxins act as enzymes has not, in fact, been confirmed in the majority of cases. Only with staphylococcal and Cl. welchii toxins are the properties of enzymes reliably found, and a substrate for their action (lecithin) identified. It may thus be accepted that the distinctive "behavior" of these toxins during the reproduction of Behring's phenomenon is associated with their distinctive type of action. This may possibly be due to the fact that the substrate of these toxins is present in large quantities in the plasma, and the toxin, when given in small doses (as in experimental conditions), acts on the dissolved lecithin and combines with it, which prevents (or attenuates) its action on sensitive cells. The significance of these properties has not been tested experimentally and we are merely putting forward a hypothesis.

It must be pointed out that, despite the absence of summation of toxic stimulation, in the experiments with staphylococcal toxin it was comparatively easy to produce summation of antigenic stimulation.

The experiments with staphylococcal toxin in fact showed that fractional administration leads to the development of immunity in at least half the animals, although the total dose of antigen injected was small (equivalent to 1-2 MLD of toxin). A single injection of such a dose of antigen (in the form of toxoid) elicits no reaction. The development of immunity thus takes place only as a result of the fractional injection of toxin, i.e. it is connected with the summation of antigenic stimulation. After the administration of tetanus toxin the opposite state of affairs is observed: It is easy to obtain summation of the toxic stimulation and impossible to obtain summation of the antigenic stimulation. We have postulated [5] that this is due to the actual amount of the substance itself in the dose of toxin injected, since antigenic stimulation may be revealed when there is an adequate amount of antigen, of which there is only little in active tetanus toxin.

It is not yet possible to explain the differences in the rate of summation of toxic stimulation. Besides the properties of the toxins themselves, many other factors may also be important; different rates of destruction or neutralization, or of excretion of the toxin from the body, the accessibility of sensitive cells and the possible rate of reaching them, the ease of compensation of the function disturbed by the toxin and the comparative strength of the toxin as a stimulus.

SUMMARY

The authors attempted to reproduce the Behring phenomenon by repeated administration of sublethal doses of staphylococcal and Cl. welchii toxins. This phenomenon could not be reproduced either with a single dose of 1/4 MLD or with a total dose exceeding 2 MLD. No cumulation of the toxin was noted. The fact that there occurred neither the development of the disease nor the death of the animals had nothing to do with the development of antitoxic immunity or the brevity of stimulation: test injections with 1-MLD resulted in the death of the animals, while more frequent administration thereof failed to provoke the development of the disease. The authors suggest

that the impossibility to reproduce Behring's phenomenon with the toxins under investigation is conditioned by their exzymatic nature and the presence of a free substrate (lecithin) in the blood plasma.

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DETERMINATION OF THE THRESHOLD OF STIMULATION OF THE SKIN RECEPTORS BY DYSENTERY AND TYPHOID ANTIGENS

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Our previous investigations showed that the injection of vaccine into the skin of the ear causes stimulation of receptors and this, when transformed into a specific process of excitation, spreads along the great auricular nerve. We studied the action potentials in the auricular nerve in response to the injection of dysentery, typhoid, coliform, paratyphoid A and B and whooping cough bacilli and streptococci. Each of these vaccines showed certain special features in the action potentials of the nerve. Analysis of the action of the vaccine showed, moreover, that the bioelectrical phenomena in the electrogram are observed not only in the intact nerve, but also in its peripheral cut end. Similar changes in the waves of action potentials in the nerve are also observed when the antigen is injected during immobilization of the animal by means of the administration of curare-like drugs and anaesthetics.

These findings suggest that antigen has the power, like many other stimuli, to cause excitation of the receptors of the skin. If this is so, then antigens, like other stimuli (chemical, thermal, mechanical, and electrical), must also have a definite threshold of stimulation.

In the present investigation our aim was to determine the threshold of stimulation of the receptors of the skin by antigen from dysentery and typhoid bacilli.

METHOD

Experiments were carried out on dogs under hexobarbital anaesthesia. The great auricular nerve was dissected out at the base of the ear, where it partially split up into branches, and its fibers were placed on electrodes. In certain cases another part of the fibers was divided and placed on a second pair of electrodes. The potentials were fed into a four-channel amplifier with symmetrical input. The action currents were recorded by means of a loop oscillograph (Siemens and Halske) on photographic paper.

The vaccines were injected into each animal strictly intradermally, at intervals of 30 min, in the upper third of the ear. Such intervals of time prevented summation of stimulation. The vaccines were injected in a dose of 0.25 ml, at room temperature (22-25°).

As the previous investigations showed, the injection of an isotonic solution of sodium chloride in the same volume and at the same temperature into the tip of the ear caused no essential changes. Different dilutions of the vaccines were used. At first, vaccine was injected containing 10,000 bacterial cells in 1 ml, and then, successively, 100,000 and 1,000,000 in 1 ml. After each injection of vaccine, the potentials were recorded for 10-15 sec at intervals of 3-5 min.

RESULTS

In the first series of experiments on 20 dogs (20 experiments), the effect of different dilutions of typhoid vaccine was studied.

After the injection of this vaccine (containing 10,000 bacterial cells in 1 ml) into the ear, no changes in the potentials in the auricular nerve were observed (Fig. 1, I).

The intradermal injection of a typhoid vaccine containing 100,000 bacterial cells caused a very slight reaction in the form of an increase in the amplitude of the oscillations.

Grouped oscillations of biphasic potentials appeared, having sharp peaks (Fig. 1, II). The reaction described usually developed soon after injection of the vaccine and lasted for 3-5 minutes.

The intradermal injection of typhoid vaccine containing 1,000,000 bacterial cells in 1 ml at once caused a considerable reaction, lasting on the average about 7 minutes. At first this was shown by single waves with an amplitude of 8-10 μv and a frequency of 5-10 per sec. After 3 minutes there appeared groups of successive oscillations with a frequency of 15-18 per second and an amplitude of 12-30 μv. After a further five minutes a continuous stream of impulses appeared, with a frequency of 18-25 per second and an amplitude of 10-20 μv (Fig. 1, III).

In a second series of experiments on 18 dogs (18 experiments) the action of dysentery vaccine was studied.

Fig. 1. Changes in the electrogram of the auricular nerve after the intradermal injection of different dilutions of typhoid antigen. Legend: a) Initial background, b) immediately after injection of antigen, c, d, e) 3, 5 and 7 minutes after injection of antigen. I) Injection of vaccine containing 10,000, II) containing 100,000 and III) containing 1,000,000 bacterial cells in 1 ml.

The experiments showed that the intradermal injection of a vaccine containing 10,000 bacterial cells in 1 ml caused no changes in the action currents in either the intact or the divided fibers of the auricular nerve (Fig. 2, I).

The intradermal injection of a vaccine containing 100,000 bacterial cells caused changes in the action

Fig. 2. Changes in the electrogram of the intact and divided auricular nerve after injection of different dilutions of dysentery antigen. Significance of the curves (from above down): electrogram of the divided nerve, electrogram of the intact nerve, time marker (50 cps); a) initial background; b) immediately after injection of antigen; c, d, e, f) 3, 5, 7 and 10 minutes after injection of antigen. Legend as in Fig. 1.

currents in the nerve as shown by the appearance of solitary and grouped waves with an amplitude of 10-15 μv and a frequency of 8-12 per second (Fig. 2, II).

The injection of a vaccine containing 1,000,000 bacterial cells caused the appearance of single and grouped waves with an amplitude of 10-15 μv, and in indi-

vidual groups up to 60-70 μv, and a frequency of 8-12 per second (Fig. 2, III). In contrast to the reaction observed after the injection of vaccine containing 100,000 bacterial cells in 1 ml, in this case a more intensive and prolonged reaction was observed, which was dependent on the strength and duration of the antigenic stimulus. The character of the changes in the potentials depended only slightly on the density of the vaccine. In the peripheral cut end of the auricular nerve, the same potentials appeared as in the intact nerve.

Antigens, like many other stimuli, thus caused stimulation of the receptors of the skin when a definite concentration was attained. Dysentery and typhoid vaccines were inactive at a concentration of 10,000 bacterial cells in 1 ml. In the presence of 100,000 bacterial cells in 1 ml, these vaccines caused excitation of the receptors and the appearance of action currents in the nerve. An increase in the concentration of the vaccine to 1,000,000 bacterial cells in 1 ml was accompanied by a more pronounced increase in the amplitude of the variations of the potentials in the nerve and a more prolonged reaction.

It is difficult at present to explain why dysentery and typhoid vaccines should have the same threshold.

More detailed investigations are necessary for this purpose. For example, by the use of more fractional dilutions than those which we used, it might be possible to obtain more accurate data regarding the true threshold of stimulation, and this might reveal different threshold values for typhoid and dysentery vaccines, which probably might vary between limits of 20-30 to 100,000 bacterial cells in 1 ml.

The negative results obtained in the experiments with injection of vaccine in a concentration of 10,000 bacterial cells in 1 ml leave no doubt about the appearance of potentials in the auricular nerve in response to the intradermal injection of the vaccine being not a side effect of the prick of the needle the stretching of the skin by the volume of fluid injected, the pain reaction, the movements of the muscles of the ear, temperature changes at the site of injection and other factors, but an expression of the stimulation of the receptors by the vaccine. This is also confirmed by the fact that the injection of physiological saline, as our previous investigations showed, does not affect the character of the background bioelectrical activity.

The appearance of impulses in the peripheral cut end of the divided afferent nerve, just as in the intact nerve, in response to the action of threshold values of antigenic stimulation, is evidence of the afferent origin of the recorded waves of bioelectrical potentials.

The ability of a vaccine to cause stimulation of receptors is evidently one of the important factors determining the development of infectious and immunological processes.

SUMMARY

Dysentery and typhoid vaccine with a definite concentration of bacterial cells may cause excitation of cutaneous receptors. Intra-dermal administration of 0.25 ml of dysentery and typhoid vaccine containing 10,000 bacterial cells per ml does not cause any excitation of receptors detectable by recording the bioelectric activity of the sensory nerve. Intra-dermal injection of typhoid or dysentery vaccine containing 100,000 bacterial cells per ml in a dose of 0.25 ml causes excitation of cutaneous receptors and biocurrents of short duration in the nerve. Intra-dermal administration of the same vaccines containing 1,000,000 bacterial cells per ml gives rise to excitation of the receptors and to the appearance of biocurrents of considerable duration and strength.

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THE DETECTION OF CLOSTRIDIUM BOTULINUM BY MEANS OF LUMINESCENT ANTIBODIES

COMMUNICATION I. THE PRODUCTION OF SPECIFIC LUMINESCENCE IN CLOSTRIDIUM BOTULINUM BY TREATMENT WITH A LUMINESCENT IMMUNE SERUM

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One of the most complicated and, at the same time, important divisions of microbiology is the rapid identification of microorganisms, both in pure culture and, in particular, when mixed with other microorganisms in different substrates. In order to solve this problem, it is possible to use the method, developed and recommended by Coons and his coworkers [5, 6], of direct detection of bacterial and viral antigens by means of luminescent immune sera. Many reports have recently appeared in the literature [7, 8, 10] of the successful use of this method for the rapid identification of a whole series of bacteria, viruses and yeasts. Much work has been done on the direct detection of various pathogenic microorganisms in objects from the external environment, such as: the causative organism of pseudoglanders [11], the typhoid bacillus [1], the streptococcus [9] and the agents of dysentery and anthrax [2, 4] and paratyphod fever [3].

In the present research, our aim was to obtain a luminescent botulinus antiserum for the identification of the microorganism Clostridium botulinum.

METHOD

In this work we used botulinus rabbit antisera, prepared by the hyperimmunization of animals with an anaculture of Cl. botulinum (strain 98 type A). In addition, normal rabbit sera were used as controls. Films were prepared in physiological saline from a suspension, in one group of experiments, of 2-day cultures of anaerobes grown on blood agar and, in the other group, from 2-day cultures grown in liquid media (casein medium, saccharose broth). The suspensions contained 5 x 10^8 bacterial cells in 1 ml. The globulin fractions were obtained from the immune and normal rabbit sera by triple precipitation with half-saturated ammonium sulfate solution and subsequent dialysis against physiological saline to remove the SO_4^{2-} ions. The protein content of the immune sera and their globulin fractions was determined by a refractometric method; an electrophoretic analysis was carried out. The immune serum contained 8.16% of protein. Its titer in the agglutination reaction was 1:300. The globulin fraction contained not less than 1% of protein, and electrophoretic examination showed absence of albumins in it. The globulin fraction was twice as active serologically as the original serum.

Conjugates of the globulins of the normal and immune sera with luminescent dyes were obtained. The dye used was liquid fluorescein isocyanate *.

Combination of the globulin with the fluorescein isocyanate was carried out by the method of Coons and Kaplan [7], in a water - dioxane - acetone medium in the cold, with constant agitation for 18 hr. In this mixture, the protein content did not exceed 2% and the dioxane content 15%; the isocyanate solution was added in a dose of 5 mg of dye to 100 mg of protein. At the end of the reaction, in order to remove the uncombined dye, the conjugate was dialyzed against physiological saline for 2-3 days; it was then precipitated 4-6 times with a half-saturated solution of ammonium sulfate, followed by dialysis to remove SO_4^{2-} ions. The precipitation was continued until the dye was completely removed from the supernatant fluid.

In order to remove nonspecific luminescence, the luminescent serum was treated with powdered liver from white mice †.

The films were stained as follows: A drop of the bacterial suspension was placed on a fat-free glass slide and dried at room temperature, after which it was fixed for 30 min in ethyl alcohol and dried in air. A few drops of undiluted luminescent immune serum were poured on to the films, placed in a humid chamber, so that the entire film was covered with serum. After 30 min the films were washed with several samples of physiological saline at pH 7.4, rinsed with distilled water and dried in air. As controls, we used films stained with luminescent normal rabbit serum. The completed films were examined beneath the MUF-ZM microscope, equipped with an OI-23

* The dye was kindly supplied by our colleague G. I. Mikhailov of the Institute of Chemical Reagents.

† We learned of the method of preparation of labeled sera in the Division of Biochemistry of the N. F. Gamaleya Institute of Immunology, Epidemiology and Microbiology.

luminescent illuminator. As a source of light we used a SVD-120A mercury lamp. Light filters SS-4, SS-8 and ZhS-17 or 2N were used, with an apochromatic objective of 90 x and an eyepiece of 7 x.

RESULTS

When the films were stained with luminescent botulinus antiserum, a specific luminescence of the Cl. botulinum cells was clearly observed, whereas the other species of microorganisms were not luminescent. The Cl. botulinum cells showed a bright green luminescence against the dark background of the film, and the morphology of the cells be distinguished. The fluorescent cells had the appearance of bacilli with rounded ends; in some films racket-shaped forms could be seen. The periphery of the cells shone more brightly than the central area.

In certain films shapeless conglomerates, showing a bright yellow or green luminescence, could sometimes be seen, but these could be differentiated without difficulty from the luminescent microorganisms because of the features of the luminescence as described above.

In order to establish the specificity of the luminescence after treatment with the luminescent botulinus antiserum, 13 strains of Cl. botulinum‡ type A, five strains of type B, three strains of type C, one strain each of types D and E, and also 38 strains of other species of microorganisms.

The experimental results, summarized in Table 1, demonstrate the specificity of the action of the luminescent botulinus antiserum. After staining with the luminescent antiserum, the Cl. botulinum cells showed a specific green luminescence, whereas if coliform, anaerobic (except Cl. botulinum), staphylococcal and anthracoid cells were treated with the same serum, no such luminescence was observed.

It must be mentioned that isolated cells of other species of bacteria possessed a hardly perceptible, pale green luminescence, but these cells resembled ill-defined formations, without characteristic morphological features. Thus the staining of Cl. botulinum cells, fixed to glass slides, with a luminescent antiserum enabled microorganisms of this species to be identified from other species: anaerobes, coliforms, staphylococci and anthracoids.

We found no essential difference in the intensity of the luminescence of all the strains which we studied of Cl. botulinum types A, B, C, D and E. In films prepared from cultures grown in liquid media, the microorganisms were more intensely luminescent than in the films from cultures grown on solid media. It must be borne in mind that tested strains differed in their ability to produce toxin. They included such highly toxic strains as strain 98 type A and strain 255 type B, as well as strains of low toxicity such as strain 13 type A and strain 346 type B, yet there was no difference in the intensity of luminescence.

In order to determine the sensitivity of the method, films were prepared from suspensions of Cl. botulinum cells of different concentrations in physiological saline. The investigation was carried out with three strains of type A and one strain of type B. Part of the film was examined under the luminescent microscope, and another part was stained by Gram's method and examined under the ordinary microscope with the same magnification.

The results given in Table 2 show that solitary Cl. botulinum cells, brightly luminescent, could be found in the films prepared from suspensions of microorganisms with concentrations of 1,000,000 and 500,000 bacterial cells in 1 ml, by luminescent microscopy.

As a result of this work, we thus succeeded in preparing a luminescent botulinus antiserum. When cultures of Cl. botulinum were stained with this serum, an intensive, characteristic luminescence of the Cl. botulinum cells was obtained. This specific luminescence may be used for the identification of this species of microorganism in microscopic preparations. When the films were treated with a luminescent normal rabbit serum, no staining of the microorganisms took place. ••

TABLE 1. Intensity of Specific Luminescence of Bacteria, Stained with Luminescent Botulinus Antiserum

Species and type of microorganism	Number of strains	Intensity of luminescence
Cl. botulinum type A	13	++++
" B	5	++++
" C	3	++++
" D	1	++++
" E	1	++++
Cl. tetani	2	—
Cl. welchii	2	—
Cl. oedematiens	2	—
Cl. histolyticum	2	—
Cl. septicum	2	—
Cl. sporogenes	2	—
Cl. putrificum	1	—
B. subtilis	4	—
B. mesentericus	3	—
B. anthracoides	2	—
Staphylococcus	2	—
E. coli	14	—

Legend: ++++ clear luminescence of bacterial cells; - absence of luminescence.

‡ Most of the strains were obtained from the L. A. Tarasevich State Control Institute for Vaccines and Sera; 14 strains of Escherichia coli were isolated in the microbiological laboratory of the Institute of Nutrition.

•• The author expresses his gratitude for the advice given by E. A. Kabanova, of the Department of Epidemiology of the N. F. Gamaleya Institute of Immunology, Epidemiology and Microbiology.

TABLE 2. Sensitivity of the Method of Staining with Luminescent Antibodies

No. of microorganisms in 1 ml of suspension	Mean no. of microorganisms in a field of vision of the ordinary microscope	Mean no. of microorganisms in a field of vision of the luminescent microscope
1	Whole field of vision	Whole field of vision
100 000 000	20	20
10 000 000	5	5
1 000 000	1 in 10 fields of vision	1 in 5 fields of vision
500 000	1 in 25 fields of vision	1 in 25 fields of vision
100 000	—	—

SUMMARY

The author obtained a conjugate of botulinus rabbit antiserum with fluorescein isocyanate, possessing immunological specificity. In staining the films of pure cultures of Cl. botulinum by the luminescent antiserum, an intensive green luminescence of these microorganisms was obtained. When preparations of other anaerobic cultures, E. coli, staphylococci or anthracoids were stained with the same conjugate, the specific luminescence was absent. In this way it is possible to reveal solitary Cl. botulinum cells in the films prepared from suspensions with a concentration of up to 500,000 bacterine cells per 1 ml. The method of staining by luminescent antibodies makes it possible to identify Cl. botulinum in microscopic preparations.

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THE PRESENCE OF A HETEROGENETIC ANTIGEN IN CROCKER'S SARCOMA

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Experimental data which indicate that by no means can all tissues of the human and animal organisms be cultivated on the chorioallantois of a chick embryo have recently accumulated.

The attempts of researchers to cultivate normal, embryonal, and pathologically altered tissues, such as the tissues of benign tumors, on this membrane have been in vain. Prolonged cultivation on the chorioallantois is only successful with malignant tumors [1, 5, 8, 12], but no explanation of this fact has yet been discovered. There are some reports [7, 10, 11] that heterotransplantation of tumors of connective-tissue origin can be more successful than that of epithelial tumors.

The antigenic affinity of the tumor and the membrane [5, 9, 11] and the low resistance of the chick embryo during the period in which a tumor is developing in it [4, 6] may be of some significance.

In our laboratory, prolonged cultivation has been done on the chorioallantois with Crocker's mouse sarcoma which had undergone up to 70 passages in a chick embryo without losing its blastomogenic properties in relation to mice.

Research on the antigenic structure of Crocker's sarcoma has shown [2] that there is a species specific antigen found in the cells of the original tumor which is identical to mouse serum proteins and which the tumor loses during the first passage to the membrane, but that the tumor does not lose its ability to be inoculated back into mice.

The purpose of this study was to further investigate the antigenic structure of Crocker's sarcoma in comparison with the antigens of the chorioallantoic membrane in order to determine whether there is or is not an antigenic affinity between them.

METHOD

The experimental object employed was Crocker's mouse sarcoma; the antigenic properties of this object were compared with the antigenic properties of the normal chorioallantois of a 17-18 day old chick embryo and with those of other mouse tissues.

The antigens from these tissues were prepared in a sterile physiological solution by the usual method [3].

Immune sera against the normal chorioallantois of a 17-18 day old chick embryo, the tissues of mouse organs, Crocker's sarcoma and Ehrlich's ascitic tumor were obtained from rabbits by the method used in our laboratory [3]. Immune sera against mouse blood serum were obtained from rabbits by the usual method of obtaining precipitating sera. The antigens for the immunization of the animals were prepared from native tissues.

The antigenic properties of the normal chorioallantoic membrane of a chick embryo were compared with those of the mouse tissues by the method employing the complement fixation reaction at + 37 deg.

All the sera were studied before the absorption of the nonspecific, incidental antibodies from them and then, if required, after their specific absorption.

RESULTS

The comparative study of the antigenic properties of three tissues - the tissues of Crocker's sarcoma, mouse spleen and the chorioallantois of a chick embryo (Table 1) – showed that the immune sera against Crocker's sarcoma, mouse spleen and the chorioallantoic membrane, studied before their absorption, reacted as strongly with the other two antigens as with the homologous antigen.

Thus the reaction of serum No. 973, obtained against Crocker's sarcoma, with the antigen from the chick embryo's chorioallantois was so strong that, in the given experiment, it was impossible to judge the specificity of serum No. 973 even from the difference in the titers of its reaction with its own and the heterogenetic antigens. Sera No. 813 and 255, obtained against the tissues of the spleen and of the chorioallantois of an 18-day old chick embryo, acted in a similar fashion.

From the data in this table, therefore, one can conclude that all three of the experimental tissues, Crocker's sarcoma, mouse spleen and the normal chorioallantois of a chick embryo, are related to each other. In view of the fact that the experimental tissues in this case are from animals of very different species, the chick embryo and the mouse, the antigen common to the mouse tissues and the chick embryo's chorioallantoic membrane is evidently heterogenetic for the two types of animals.

In spite of the considerable reaction of the sera against the mouse tissues with the heterogenetic antigen from the chick embryo's chorioallantois and of the serum

TABLE 1. The Antigenic Affinity Between the Tissues of a Chick Embryo's Chorioallantoic Membrane and the Tissues of Crocker's Sarcoma and Mouse Spleen

Serum No.	Serum dilution	Absorbent	Results of complement fixation reaction with antigens from the tissues of: Crocker's sarcoma	spleen	chorioallantois
973 Against Crocker's sarcoma	1:40	—	++++	+++	++++
	1:80		++++	++(+)	++++
	1:160		++++	+(+)	++++
	1:320		+(+)	±	±
813 against spleen	1:40	—	++++	++++	++++
	1:80		++++	++++	++++
	1:160		++++	++++	++++
	1:320		++++	++++	+++
255 against membrane	1:40	—	++++	++++	++++
	1:80		+++ +	++++	++++
	1:160		++++	++++	++++
	1:320		++++	++(+)	++++
973	1:40	Chorioallantoic membrane	++++	±	—
	1:80		++	—	—
	1:160		—	—	—
	1:320		—	—	—
813	1:40	Crocker's sarcoma	+++	++++	+
	1:80		+	++++	—
	1:160		—	++++	—
	1:320		—	++++	—
255	1:40	Crocker's sarcoma	+	±	++++
	1:80		—	—	+++(+)
	1:160		—	—	±
	1:320		—	—	—

Key: ++++, +++(+), +++, ++(+), ++, +(+), + show different degrees of a positive complement fixation reaction; ± shows a doubtful reaction, - shows a negative reaction.

against the chorioallantois with the antigens from the mouse tissues, these sera could be subjected to specific absorption, and the incidental, nonspecific antibodies could be removed from them.

Table 1 also gives typical records of the experiments with the absorption of these sera by formalin-treated tissues in order to remove nonspecific antibodies. It is evident from this table that when the immune serum against Crocker's mouse tumor was absorbed by the tissue of a chick embryo's chorioallantoic membrane, the antibodies to the membrane were completely eliminated, but the serum in this case kept its ability to react mainly with the antigen against which it was obtained.

Absorption of the serum against the chorioallantoic membrance by Crocker's tumor in the same way eliminated nonspecific antibodies to the mouse tissues, but left the antibodies to the antigens from the chorioallantoic membrane.

Therefore, the heterogenetic antigen contained in the tissues of mouse spleen and Crocker's sarcoma as well as in the tissue of a chick embryo's chorioallantois is an incidental antigen, accessory to the specific antigens contained in these tissues.

The presence of the heterogenetic antigen in the tissues of mouse spleen and Crocker's sarcoma made it necessary to investigate the question of whether a heterogenetic antigen common to the chorioallantois of a chick embryo exists in other mouse organs.

Table 2 presents experimental records obtained in the investigation of serum No. 254, obtained against the normal chorioallantois of a chick embryo, and the antigens from the tissues of mouse organs in the complement fixation reaction. This table also includes the records from the investigation of immune sera against the antigens from the mouse tissues with water-salt extracts of the normal chorioallantois of a chick embryo. In this case, all the sera were studied before absorption.

The data in Table 2 show that the immune sera obtained against mouse liver and brain and those against mouse blood serum and the cells of Ehrlich's ascitic adenocarcinoma did not react with the antigen from a chick embryo's normal chorioallantoic membrane. In the same way, the serum against the chorioallantois did not react with the antigens from the above tissues.

One can therefore conclude that mouse blood serum, liver and brain, as well as the cells of Ehrlich's ascitic

TABLE 2. The Lack of Antigenic Affinity Between the Tissues of a Chick Embryo's Chorioallantois and Certain Mouse Tissues

Serum No.	Serum dilution	Results of reaction with antigens			Serum No.	Serum dilution	Results of reaction with antigens			Serum No.	Serum dilution	Results of reaction with antigens	
		embryo membrane	mouse liver				embryo membrane	mouse brain				embryo membrane	mouse serum
254 against membrane tissue	1:40	++++	–	254 against membrane tissue	254 against membrane tissue	1:40	++++	–	254 against membrane tissue	254 against membrane tissue	1:40	++++	–
	1:80	++++	–			1:80	++++	–			1:80	++++	–
	1:160	++++	–			1:160	+++	–			1:160	+++	–
	1:320	+++	–			1:320	+++(+)	–			1:320	++(+)	–
225 against liver tissue	1:40	–	++++	612 against brain tissue	248 against mouse serum	1:40	–	++++	248 against mouse serum	893 against Ehrlich's carcinoma	1:40	–	++++
	1:80	–	++++			1:80	–	++++			1:80	–	++++
	1:160	–	++++			1:160	–	+(+)			1:160	–	++++
	1:320	–	±			1:320	–	–			1:320	–	++++

Serum No.	Serum dilution	Results of reaction with antigens	
		embryo membrane	Ehrlich's carcinoma
254 against membrane tissue	1:40	++++	–
	1:80	++++	–
	1:160	+++	–
	1:320	++(+)	–

Note: Symbols mean the same as in Table 1.

carcinoma, do not have the heterogenetic antigen common to the chorioallantois of a chick embryo. The nature of this antigen and its relation to the heterogenetic Forssman's antigens is not yet clear.

As to the two experimental mouse tumors, Ehrlich's ascitic carcinoma does not contain a heterogenetic antigen identical to the antigen found in the chick embryo's chorioallantoic membrane, but Crocker's sarcoma does

contain such an antigen. It is known from the literary data [1, 10] that cultivation of Ehrlich's carcinoma on the chorioallantois of a chick embryo is less successful than cultivation of Crocker's sarcoma. One can propose that the presence of a common heterogenetic antigen in Crocker's sarcoma and the chorioallantois of a chick embryo is one of the factors aiding the successful prolonged cultivation of this tumor after heterotransplantation on the chorioallantois of a chick embryo.

SUMMARY

The author investigated the antigenic properties of Crocker's sarcoma in mice and of the chorioallantoic membrane of a chick embryo. A common heterogenic antigen was soon to be present in the chorioallantoic membrane of a chick embryo in the Crocker's mouse sarcoma and in the mouse spleen. The tissues of mouse liver and brain and the mouse blood serum, as well as the cells of Ehrlich's ascitic adenocarcinoma do not contain this antigen.

It may be suggested that the affinity of the tumor and the membrane with respect to the heterogenetic antigen is one of the factors aiding successful prolonged cultivation of Crocker's tumor on the chorioallantoic membrane of a chick embryo without loss of its blastomogenic properties in mice.

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THE EFFECT OF SARCOLYSIN AND DOPAN ON THE BIOSYNTHESIS OF NUCLEIC ACID PYRIMIDINES IN TRANSPLANTED TUMORS AND IN THE TISSUES OF THE RECIPIENT

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It has been shown [1, 3, 5] that the nucleic acid metabolism in the tissues of rats and mice undergoes certain essential changes during the regression of transplanted tumors under the influence of chemotherapeutic drugs.

In order to study the mechanism of these changes, with the assistance of labeled ureidosuccinic acid we investigated changes in the biosynthesis of the nucleic acid pyrimidines in transplanted tumors and in the tissues of recipient rats in response to the action of antitumor drugs, namely sarcolysin and dopan.

Sarcolysin [di-(2-chloroethyl) aminophenylalanine], when administered to rats with sarcoma 45 in certain doses, brings about complete absorption of this sarcoma [4]. A dose of 30 mg/kg body weight is toxic, and leads to death of rats on the 4th-5th day after administration. When performing experiments with sarcolysin in vivo we selected this dosage, in order to secure the maximum possible action of the drug on the processes under investigation. Dopan [4-methyl-5-di(2-chloroethyl) aminouracil] causes lasting absorption of sarcoma 45 in 90% of rats so treated [2].

In experiments in vitro we gave sarcolysin and dopan in doses considerably larger than therapeutic, in order to exceed the threshold of ineffective combination of the drugs with the tissue.

METHOD

Ureidosuccinic acid (US), with the ureide carbon labeled with C^{14}, was synthesized by the method of Nyc and Mitchell [6]. The relative activity of the US thus obtained was 1.9 mC.

For the experiments we selected male rats weighing up to 110 g. Sarcolysin and dopan were given on the 12th day after transplantation of a sarcoma 45. Sarcolysin was injected intraperitoneally as a single dose of 30 mg/kg. Dopan was given by mouth in the form of a suspension in 1% starch solution, in three doses of 0.75 mg/kg body weight at intervals of 72 hr. The animals were sacrificed 72 hr after the third dose. Radioactive US was injected subcutaneously or intraperitoneally in a neutral aqueous solution.

After a single injection of US into the rats the radioactivity per gram body weight was 7000-10,000 cpm; after repeated injection at intervals of three and 18 hours between injections, in order to produce gradual accumulation of radioactivity in the animals, the total dose of radioactivity injected was 25,000-28,000 cpm/g body weight. The radioactivity of the specimens was measured by means of the MST-17 end-type counter and a B-2 apparatus. Animals were sacrificed 2 hr after injection of US.

The tissues for investigation were treated by the method of Schmidt and Thanhauser [7]. A weighed sample of tissue was minced and ground up with five times its own weight of 5% trichloroacetic acid (TCA) or with 0.2 N $HClO_4$. The radioactivity of the supernatant fluid obtained after centrifugation (subsequently called the acid-soluble fraction) was determined after neutralization and suitable treatment. The lipids were extracted from the precipitate; the residue, containing nucleoproteins, was dried with alcohol and ether, and analyzed, and hot extraction of the nucleic acids in a separate portion of the residue was carried out with 5% TCA or 0.2% $HClO_4$.

The results obtained are shown either as a ratio between the radioactivity detected in impulses (x 100) per 10 mg of the specimen and the radioactivity injected per gram body weight, or as impulses per 10 mg of specimen. The action of the antitumor preparations was investigated in vitro and in vivo.

RESULTS

Effect of sarcolysin in vitro. These experiments were carried out on sections of liver and sarcoma 45, obtained from rats on the 12th day after transplantation. In the experiment we used 0.5 g of tissue sections, sarcolysin solution in a dose of 100 mg/kg and US solution with an activity of 100,000 impulses to a test sample of 5 ml. The samples were incubated in Krebs-Ringer solution for 60-90 min in a water bath at a constant temperature of 37°, with agitation.

TABLE 1. The Effect of Sarcolysin on the Incorporation of US in the Nucleoproteins of Sections of Liver and Sarcoma 45 in 45 Rats (in impulses per 10 mg of the specimen)

Experiment No.	Liver		Sarcoma 45	
	control	experiment	control	experiment
1	68	74	113	103
2	79	75	158	138
3	57	62	89	67
4	52	48	83	75
5	—	—	34	30

It will be seen from the figures in Table 1 that sarcolysin had no effect on the incorporation of US by the liver sections of the recipient rats. The effect of sarcolysin on the incorporation of US by the nucleoproteins of the sections of sarcoma 45 was slight, although in the five experiments illustrated it was shown in the same direction, namely as a lowering of the intensity of incorporation on the average by 10%.

Effect of sarcolysin in vivo. Sarcoma 45 was transplanted into rats, and the animals then received sarcolysin by intraperitoneal injection in a dose of 30 mg/kg. The animals were sacrificed three and 24 hours after the injection of sarcolysin and two hours after injection of US. Control rats, in which tumors had been transplanted, were sacrificed at the same times (Table 2).

TABLE 2. The Effect of Sarcolysin on the Incorporation of US in the Nucleoproteins of the Tissues of Rats with Sarcoma 45 (24 hours after injection)

Tissues	Nucleoproteins		Acid-soluble compounds		Nucleic acids	
	control	experiment	control	experiment	control	experiment
Liver	239 0,94	361 1,46	292 1,14	433 1,75	180 0,71	240 1,00
Mucous membrane of the small intestine	57 0,22	40 0,16	48 0,18	13 0,05	16 0,06	16 0,06
Sarcoma 45	17 0,07	26 0,10	16 0,06	16 0,06	7 0,02	8 0,03

Note: Upper row of figures — impulses per 10 mg of specimen; lower — relative activity.

In Table 2 are shown the mean results of three experiments carried out on rats in which the same tumor was transplanted. It follows from these results that an obvious increase in the intensity of biosynthesis of pyrimidines from US, of roughly 25-34%, took place in the liver under the influence of sarcolysin in all the fractions investigated. In the mucous membrane of the small intestine the absolute values of the radioactivity were small, although it could be noticed that the incorporation of US into the fractions of nucleoproteins and acid-soluble compounds was diminished under the influence of sarcolysin. Practically no changes were found in sarcoma 45.

At sacrifice of the rats three hours after injection of sarcolysin, the changes in these particular tissues were in the same direction as at the investigation after 24 hours.

In rats with ascitic hepatoma (Zazhdel' strain), after injection of sarcolysin in a dose of 30 mg/kg into the ascitic fluid, 22 hours after injection an increase in the incorporation of US into the nucleoproteins of the liver by an average of 40 was also observed (Table 3).

TABLE 3. The Effect of Sarcolysin on the Incorporation of US in the Nucleoproteins of the Liver and Ascitic Fluid of Rats (22 hours after injection)

Tissues	Nucleoproteins	
	control	experiment
Liver	304	420
Ascitic fluid .	43	35

Note: In impulses per 10 mg of specimen. Mean of four experiments

TABLE 4. The Effect of Dopan on the Incorporation of US in the Nucleoproteins of Sections of Liver and Sarcoma 45

Experiment No.	Liver		Sarcoma 45	
	control	experiment	control	experiment
1	18	18	58	54
2	21	18	60	50
3	20	24	50	60
4	18	24	—	—

Note: In impulses per 10 mg of specimen.

Effect of dopan. The elucidation of the effect of dopan on the biosynthesis of pyrimidines from US was of especial interest, for dopan is a chloroethylamino-derivative of uracil, and it might have been expected that during interaction with US, the precursor of the pyrimidines, competitive relationships might have arisen during the synthesis of nucleic acids. Experiments were carried out in vitro on sections of liver and sarcoma 45. Dopan was added in a dose of 100 mg/kg body weight (0.05 mg dopan corresponding to 500 mg of sections) in the form of a suspension in Krebs-Ringer solution. US

TABLE 5. The Effect of Dopan on the Incorporation of US in the Nucleoproteins of the Tissues of Rats with Sarcoma 45

Tissue	Nucleoproteins		Acid-soluble compounds	
	control	experiment	control	experiment
Liver	314 1,44	436 1,89	347 1,58	525 2,26
Mucous membrane of the small intestine	54 0,24	61 0,26	— —	— —
Sarcoma 45	31 0,14	55 0,23	46 0,21	73 0,31

Note: Upper row of figures -- impulses per 10 mg of specimen; lower -- relative activity.

with a total radioactivity of 65,000 cpm was added to the 5 ml samples.

Incubation was for one hour in a water bath at a constant temperature of 37° with agitation (Table 4).

It may be seen from the figures in Table 4 that in our experimental conditions in no case had dopan any effect on the incorporation of US in the nucleoproteins of the sections of liver and sarcoma 45.

In the experiments in vivo, dopan was given on three occasions, as described in the section on method. The results obtained are shown in Table 5.

The dopan experiments on intact animals showed that the same intensification of biosynthesis of the pyrimidines, of the order of 35-40%, takes place in the nucleoproteins of the liver under the influence of dopan as was observed under the influence of sarcolysin. It was also found that the incorporation of US in the nucleoproteins of sarcoma 45 is increased as a result of the action of dopan.

The information now available on the mechanism of action of sarcolysin and dopan (only part of which has been published [3]) supports the point of view that injury of the nucleic acid metabolism is one of the main, but probably not the only, links in the mechanism of action of antitumor drugs with chloroethylamino-groups. Whereas the action of antitumor drugs of the antimetabolite group is characterized by their action on a particular and narrowly specific link in metabolism, that of the drugs under investigation, with an alkyl group, is evidently characterized by a wide field of interaction with many links in metabolism.

According to our findings, sarcolysin and, what is especially interesting, dopan do not lead to any specific injury to the biosynthesis of pyrimidine-nucleic acids from US as a precursor.

SUMMARY

The author studies the effect of sarcolysin and dopan on the synthesis of nucleic acid pyrimidines from the ureidosuccinic acid (with C^{14}-labeled ureide carbon) in the tissues of rats with sarcoma 45 and ascitic hepatoma. The incorporation of ureidosuccinic acid in the nucleoproteins of the liver is increased by sarcolysin. The utilization of ureidosuccinic acid by sarcoma 45 and by the cells of the ascitic tumor during the action of sarcolysin and dopan is changed only slightly.

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THE IMMUNOLOGICAL PROPERTIES OF CERTAIN FRACTIONS OF EHRLICH'S ADENOCARCINOMA OF MICE

COMMUNICATION I. THE STUDY OF THE ANTIGENIC COMPOSITION OF TUMOR FRACTIONS BY THE METHOD OF ANAPHYLAXIS WITH DESENSITIZATION

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The study of the specific antigens and their location in the tumor cell is of great importance in the diagnosis of tumors and vaccination against them. Research in the last few years [1-4] suggests that serological activity is present in the globulin, microsomatic and other fractions of the tumor. The vaccinal activity of the serologically active fractions has, however, received very little study. In connection with the isolation of "protective antigens" - globulin fractions of the microorganisms of anthrax and plague [5, 6, 8, 9], which possess high vaccinal activity - interest in chemical vaccination has increased considerably.

The aim of the present research was to compare the serological and vaccinal activity of different tumor fractions, some of which were obtained by methods used to isolate the "protective antigens" of microorganisms. In this communication we give the results of a study of the antigenic composition of the tumor fractions.

EXPERIMENTAL METHOD

As an experimental model we used a transplanted strain of Ehrlich's mouse adenocarcinoma.

Fractionation of the tumor material. Fresh, washed ascitic cells were homogenized in a cold 0.14 M solution of NaCl. After centrifugation at 2500 rpm for 15 minutes, the fractions precipitated at 33, 46 and 50% saturation were successively salted out with ammonium sulfate at +4°. The last fractions, precipitated at 46 and 50% saturation, were called globulin fractions I and II respectively. The protective antigen of the microorganism of anthrax was also obtained from a filtrate of a culture at 46 and 50% saturation with ammonium sulfate [6, 8].

Fraction III was isolated from the tumor extract in 0.14 M NaCl by means of precipitation with potassium aluminum alum, in accordance with the technique used [8] for isolation of the anthrax protective antigen. A 10% solution of potassium aluminum alum was added to the saline extract to give a final concentration of 0.1%; 10% acetic acid was added to bring the pH to 5.9. The precipitate which formed overnight at +4° was collected by centrifugation, dissolved in cold 0.2 M citric acid and precipitated with 2.5% trichloroacetic acid. The fraction III thus obtained was called the alum precipitate.

Tumor homogenate in cold 0.14 M NaCl solution with 0.01 M sodium citrate was twice centrifuged (8000 rpm) for 20 minutes at +4°. The precipitate was homogenized in an equal volume of 2 M NaCl. The extraction took place while the mixture was allowed to stand overnight at +4°. After centrifugation (8000 rpm) for an hour at +4°, the supernatant fluid was poured into cold distilled water to give a final concentration of 0.14 M NaCl. The threads thus formed were transferred to a 1 M solution of NaCl. Fraction IV was given the name of nucleoprotein.

The residue remaining after extraction with 1 M NaCl was extracted with 0.25% NaOH overnight at +4°. After centrifugation, the supernatant fluid was acidified with dilute acetic acid to pH = 5.9. The precipitate forming overnight at +4° was separated by centrifugation. Fraction V was called alkaline extract.

Fractions I, II, III and IV were dialyzed against physiological saline. All the fractions were lyophilized and kept in sealed ampoules at +4°.

Serological activity of the fractions. In order to detect the specific and species antigens in the fractions obtained, we used the reaction of anaphylaxis with desensitization. In the first series we studied the content of specific antigen in the fractions.

Guinea pigs weighing 220-250 g were sensitized subcutaneously with washed ascitic cells in a dose of 5.2 mg of protein (according to Kjeldahl's method) per animal. Three weeks after sensitization, intraperitoneal desensitization was carried out with 1 ml of normal antigen (a mixture of mouse serum and a saline extract of

the skin of the abdomen of a female mouse in proportions of 1:3) in a dose of 20 mg dry weight. Next day, after testing for completeness of desensitization to normal antigen, as an assaulting antigen solutions of fractions I, II and V of the tumor were injected. All the antigens were assayed according to dry weight of the lyophilized preparation, and injected into the heart.

EXPERIMENTAL RESULTS

The results obtained are shown in Table 1.

The sensitized guinea pigs responded by an anaphylactic reaction to the injection of globulin fractions I and II. This suggests the presence in the globulin fractions of a specific tumor antigen, absent from normal tissues. Injection of the alkaline extract of the tumor (fraction V) did not elicit a response reaction. It is evident that treatment with alkali appreciably lowered the antigenic properties of the fraction thus obtained.

In the second series of experiments we studied the distribution of the species antigen in the tumor fractions. Guinea pigs were sensitized subcutaneously with solutions of fractions I, III, IV and V of the tumor in a dose of 5 mg dry weight per guinea pig. Three weeks later, a solution of lyophilized mouse serum was injected as a species antigen. In order to reveal any common antigenic factor in the fraction and the initial tumor extract, a saline extract of the tumor was injected. All antigens were injected in a dose of 0.2-0.3 ml into the jugular

TABLE 1. Detection of Specific Antigen in Fractions of Ehrlich's Adenocarcinoma by the Reaction of Anaphylaxis with Desensitization

Sensitization		Second desensitization[1]		Test of complete desensitization		Assaulting injection		
ascitic cells of Ehrlich's adenocarcinoma		normal antigen				fractions of Ehrlich's adenocarcinoma		
guinea pig no.	dose of protein (mg)	dose of protein (mg)	reaction	dose of protein (mg)	reaction	antigen	dose of protein (mg)	reaction
15	5,2	—	.	12	—	Saline extract	12	++
18	5,2	—	.	9	—		8	+
57к	Control	—	.	12	—		8	—
58к	»	—	.		.		8	—
59к	»	—	.		.		8	—
2	5,2	2	+	10	—	Globulin fraction I	10	++
4	5,2	—	.	4	±		10	++
16	5,2	—	.	12	—		10	+
3	5,2	2	—	10	±		10	±
56к	Control	—	.	12	—		10	—
48к	»	—	.	10	—		10	—
17	5,2	—	.	7,5	—	Globulin fraction II	10	++
12	5,2	—	.	5	—		10	++
11	5,2	—	.	10	—		10	+
53к	Control	—	.	—	.		10	—
55к	»	—	.	—	.		10	—
55к	»	—	.	—	.		10	—
5	5,2	—	.	10	±	Alkaline extract (fraction V)	10	—
6	5,2	—	.	10	—		10	—
8	5,2	—	.	10	—		10	—
7	5,2	—	.	10	—		10	—
50к	Control	—	.	10	—		10	—
51к	»	—	.	10	—		10	—
52к	»	—	.	10	—		10	—

Legend: — no reaction; + brief scratching; ++ strong scratching, sneezing, shortness of breath, dishevelling of the fur, coughing; +++ the same but more marked, and excretion of urine and feces; ++++ convulsive jumps, fits, terminating in death of the animal; ± doubtful reaction; · no antigen injected.

[1]The first desensitization was carried out intraperitoneally the day before the experiment.

TABLE 2. Detection of Species Antigen in Fractions of Ehrlich's Adenocarcinoma by the Reaction of Anaphylaxis with Desensitization

Guinea pig no.	Sensitization: fractions of Ehrlich's adenocarcinoma, dose 5 mg dry weight	Desensitization: normal mouse serum, dose (mg)	Desensitization: normal mouse serum, reaction	Test for complete desensitization: normal mouse serum, dose (mg)	Test for complete desensitization: normal mouse serum, reaction	Assaulting injection: saline extract of Ehrlich's mouse adenocarcinoma, dose (mg)	Assaulting injection: saline extract of Ehrlich's mouse adenocarcinoma, reaction
31	Globulin fraction I	10	++++ Death	.	.	.	.
35		10	++++ Death	.	.	.	.
35		10	+++	10	±	15	++
32		5	++	10	±	15	++
33		5	++	10	—	15	+
26	Alum precipitate (fraction III)	10	++	10	—	15	+++
25		10	+	10	±	15	±
28		10	+	10	—	15	+
24		10	±	10	—	15	+
27		10	+	10	—	15	+++
21	Alkaline extract (fraction V)	20	—	30	—	16	++
20		20	+	30	±	16	+
19		20	+	30	±	16	+
23		20	—	30	—	16	+
36	Nucleoprotein (fraction IV)	5	+	15	—	15	±
37		10	+	15	—	15	±
38		10	±	15	—	15	±
40		10	—	15	—	15	±
39		10	—	15	—	15	—
41	Control	.	.	16	—	.	.
43		.	.	15	—	.	.
44		.	.	15	—	.	.

Legend as in Table 1.

vein of the animal. It can be seen from Table 2 that the majority of the sensitized guinea pigs responded by an anaphylactic reaction to the injection of normal mouse serum. The most marked reaction was observed in the guinea pigs sensitized with globulin fraction I. Animals sensitized with alum precipitate of the ascitic fluid (fraction III) reacted more weakly to the species antigen. This was evidence of the presence of a species antigen in fractions I and III. A very weak reaction was shown by guinea pigs sensitized with the nucleoprotein and alkaline extracts of the tumor (fractions IV and V).

A clear reaction to injection of the saline extract was noted in the guinea pigs sensitized with globulin fraction I and alum precipitate of ascitic fluid. An appreciable reaction was observed in the animals sensitized with alkaline extract of the tumor. This showed that in fractions I, III and V are present antigens which are different from the species antigens, probably tissue or specific antigens.

The study of the antigenic properties of the fractions obtained by means of the anaphylaxis reaction thus showed the presence of specific antigens in the globulin fractions and species antigens in the globulin and alum precipitate fractions.

The results of our experiments are confirmed by reports in the literature demonstrating the presence of specific [2, 4, 10] and normal [7] antigens in the globulin fractions of a tumor.

SUMMARY

The author obtained a number of fractions of Ehrlich's mouse adenocarcinoma by the procedure used for the iso-

lation of the protective antigens of microorganisms. The antigenic properties of the fractions were studied by means of anaphylaxis with desensitization. Specific and species antigens were revealed in the globulin fractions of the tumor.

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THE ACTION OF SARCOLYSIN ON REGENERATION IN AMPHIBIA AND ON TUMORS IN MICE UNDER SLIGHT HYPOTHERMIA

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Investigation of the action of novoembichin [2-chloropropyl-di(2-chloroethyl) amine] and TET (2,4,6-triethylenimino-1,3,5-triazine) on regeneration of the tail of tadpoles after partial amputation showed [4] that these compounds, when injected intraperitoneally, cause considerable inhibion of the regeneration process; their inhibitory effect is, however, considerably weakened if the animals are kept in colder water (14-16°) than that required for optimal conditions of regeneration of the tail (18-22°).

Sarcolysin [p-di(2-chloroethyl) aminophenylalanine], like novoembichin, is a derivative of chloroethylamine, but differs from the latter by having an inhibitory action many times more powerful on the growth of tumors in animals [2]. The inhibitory action of sarcolysin on the reparative regeneration of the liver of the rat is, however, expressed far more weakly than that of other chloroethylamine derivatives [15].

In the present research we studied the distinctive features of the biological action of sarcolysin on the processes of regeneration and growth.

METHOD

Part (1/5) of the tail was amputated in stage II tadpoles of Rana temporaria and Rana ridibunda and, at the same time, the drug was injected intraperitoneally by a method previously described [4], in one single large dose, amounting to not less than 2/5 the lethal dose per kg body weight. In a control group the tail was also amputated, but no drug was injected.

Observations on regeneration lasted 8-10 days. Every 2-3 days the length of the regenerate was measured under the loupe with an ocular micrometer, and the mean value for each group of 25 animals was calculated. These mean values were used to construct a graph of growth and, at the end of the experiment, the percentage inhibition according to the mean length of the regenerate was calculated, i. e. the difference between the mean length of the regenerates in the control and experimental animals, expressed as a percentage of the mean length of the regenerate in the control tadpoles. As an additional control, and in order to compare the action of sarcolysin with that of novoembichin and TET, which had previously been studied on this same experimental object, experiments with these two drugs were conducted at the same time. Altogether 14 experiments were carried out.

RESULTS

In contrast to novoembichin and TET, sarcolysin acts very weakly on regeneration of the tail in ordinary conditions, optimal for the regeneration of this organ. Sarcolysin caused from 11 to 20% inhibition of growth of the regenerate, whereas novoembichin and TET, in parallel experiments with a common control, caused 50-60% inhibition (Fig. 1, 1).

When, however, the midday temperature was lowered by 3-6°, i.e. in cold weather, or when the animals were kept in a refrigerator (at +10°), the inhibitory effect of all three drugs showed obvious changes. Novoembichin and TET almost completely lost their growth-inhibiting properties, and caused about 10% inhibition, whereas sarcolysin, on the other hand, increased its inhibition of growth to from 50 to 80% (Figs. 1, 2 and 3).

It may be postulated from these results that, in animals without a developed thermoregulatory system, the slowing of the metabolic processes by a lowering of the environmental temperature weakens the inhibitory effect of novoembichin and TET on the processes of proliferation (as has been pointed out earlier [4]) and, conversely, increases the inhibitory action of sarcolysin.

An attempt was then made to verify if the relationships concerning the action of these drugs, as shown by a study of regeneration in amphibia, could be applied to the action of the same drugs on the growth of tumors in

* The experiments on tadpoles were conducted at the biological station of the Laboratory of Growth and Development (Head – Prof. L. D. Liozner) of the Institute of Experimental Biology (Dir. – Prof. I. N. Maiskii) of the AMN SSSR.

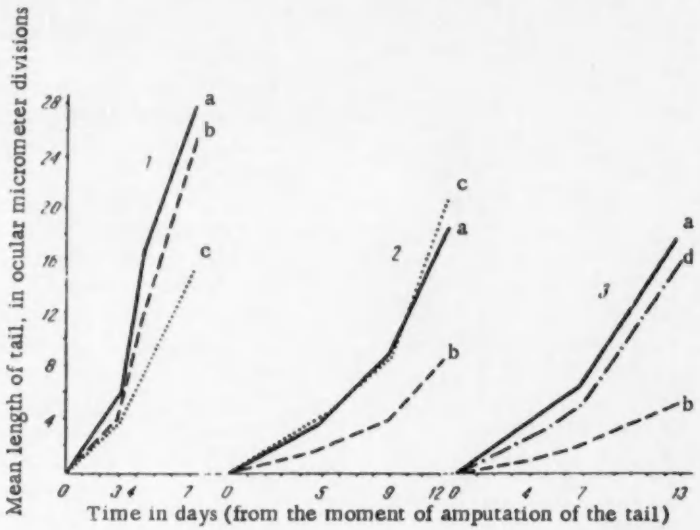

Fig. 1. Dynamics of growth of the regenerating tail of the tadpole of Rana temporaria. a – Control; b – after injection of sarcolysin; c – after injection of novoembichin; d – after injection of TET. 1 – at a midday water temperature of 21°. Doses: sarcolysin 150 mg/kg, novoembichin 6 mg/kg; 2 – at a temperature of 14°. Doses: sarcolysin 50 mg/kg, novoembichin 8 mg/kg; 3 – at a temperature of 12°. Doses: sarcolysin 80 mg/kg, TET 10 mg/kg. I) Mean length of tail, in ocular micrometer divisions II) Time in days (from the moment of amputation of the tail).

mammals. For this purpose an attempt was first made to obtain a strain of Ehrlich's tumor, the cells of which could be adapted more or less permanently to the slower metabolism of a subnormal temperature. Ascitic cells from an Ehrlich's tumor, mixed with citrate, were accordingly kept at 4° for 30 days, after which the mass of cells which settled to the bottom was washed and diluted with physiological saline and then inoculated into mice. The inoculation was carried out intramuscularly into the thigh on one side, and into the thigh of the opposite limb of the same mouse was injected normal, fresh Ehrlich's ascitic cells. It was found that cells which had been subjected to prolonged cooling, like normal cells, caused the development of tumors in 100% of cases, but the tumors were more compact and slower growing than normal. Treatment began on the fifth day after inoculation of the tumor and continued for ten days. Every three days the size of the tumors was measured and the mean tumor diameter for the whole series calculated. From the mean values obtained a graph of tumor growth in the experimental and control animals was constructed (Fig. 2). The degree of inhibition of growth of the tumors was determined as the difference between the mean weight of the tumors in the control and experimental animals, expressed as a percentage of the mean weight of the control tumors. The controls were untreated animals which, like the experimental, received inoculations of normal and cooled strains.

The experiments in which both types of tumors were treated with sarcolysin and TET gave results which were identical in principle with those obtained for these drugs on the regenerating tail in tadpoles. TET, which was injected in a sublethal dose of 3 mg/kg (since in a therapeutic dose it does not generally inhibit growth of an Ehrlich's tumor inoculated intramuscularly), caused

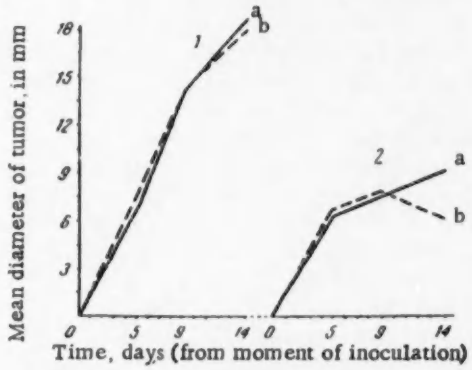

Fig. 2. Dynamics of growth of an Ehrlich's tumor, inoculated intramuscularly. a – Control; b – after injection of sarcolysin (3 mg/kg every 48 hr). 1 – normal strain; 2 – strain treated by cooling. I) Mean diameter of tumor, in mm. II) Time, days (from moment of inoculation).

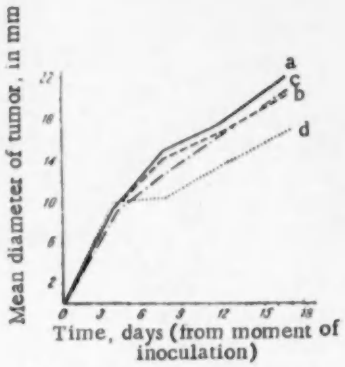

Fig. 3. Dynamics of growth of an Ehrlich's tumor, inoculated intramuscularly. a – Control; b – after injection of sarcolysin (4 mg/kg every 72 hr); c – after injection of chlorpromazine (20 mg/kg daily); d – after injection of chlorpromazine and sarcolysin in the same doses. 1) Mean diameter of tumor, in mm. 2) Time, days (from moment of inoculation).

inhibition of the growth of tumors of the normal strain by 59% and of the strain adapted to cold by 30%. In a therapeutic dose (3 mg/kg every 48 hr), sarcolysin caused the usual slight inhibition for a tumor of the normal strain when inoculated intramuscularly (15%), and on the other hand, a more considerable inhibition (53%) of the adapted strain (see Fig. 2).

When the metabolic processes of the tumor were retarded by adaptation to cold, sarcolysin thus also acquired the power to inhibit the growth of this tumor to a considerable degree, but TET under the same conditions lost this power to a large extent.

In order to potentiate the action of sarcolysin on the tumors we also made use of pharmacological hypothermia of the animal as a whole by means of the injection of chlorpromazine [N(3-dimethylaminopropyl)-2-chlorophenothiazine], which in large doses causes inhibition of oxidative processes in the tissues and a fall in the body temperature of animals for a few hours [8, 10, 11, 13]. On this account, chlorpromazine probably possesses a weak antitumor action [7].

The ordinary course of injections of sarcolysin, after the preliminary injection of large doses of chlorpromazine (20 mg/kg daily, subcutaneously), resulted in 45% inhibition of growth of the tumors in mice with an Ehrlich's tumor inoculated intramuscularly, i.e. more than twice the effect produced by sarcolysin and chlorpromazine when given separately (Fig. 3).

From a comparison of our results with those in the literature on the action of the chloroethylamines and ionizing radiation on the cell, it may be surmised that the activity of the antitumor drugs investigated is dependent on the state of the oxidative processes in the tissues. A subnormal environmental temperature, in the case of cold-blooded animals [9, 12], and chlorpromazine, in the case of mammals [8, 10, 11, 13], are known to lower the intensity of the oxidative processes in the tissues. In accordance with these findings it is possible that the inhibitory action of sarcolysin in our experiments (during hypothermia) is strengthened as a result of the slight anoxia of the tissues of the regenerate or tumor, whereas that same degree of anoxia is evidently a factor protecting these tissues from the action of novoembichin and TET, just as has been shown by several workers in the case of ionizing radiation [1, 3, 5, 6, 14]. Our findings thus show that sarcolysin differs to a certain extent by its mechanism of action from novoembichin and TET; this probably depends on the presence of a natural group (phenylalanine) in the sarcolysin molecule. In the experiments with novoembichin and TET we were possibly confronted by a special phenomenon resembling the oxygen effect in radiobiology, and confirming the radiomimetic character of the action of these drugs on the cell. In contrast to novoembichin and TET, sarcolysin revealed distinctive features of its biological action that were not typical of radiomimetic substances and ionizing radiation.

SUMMARY

A comparative effect of the action of sarcolysin, novoembichin and TEM on the regeneration of the tadpole tail was studied both at the optimal and at subnormal temperatures (3-6° lower). Slowing of the metabolism provoked by the temperature reduction intensified the inhibitive action of sarcolysin on the tail regeneration, but weakened the effect of novoembichin and TEM. The author also investigated the action of sarcolysin and TEM on Ehrlich's tumor (transplanted into mice intramuscularly)-both on the usual strain and a strain subjected to prolonged chilling at 4°C. The inhibitive effect of sarcolysin on the tumor growth of the chilled strain was 3-4 times greater than on that of the normal strain. Conversely, the inhibitive effect of TEM on the growth of the normal strain tumors was double that exercised on the chilled tumors.

The combined effect of sarcolysin and chlorpromazine on a normal strain Ehrlich's tumor was also investigated. In this case the inhibitive effect of sarcolysin on the tumor was also intensified by the pharmacological hypothermia.

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NEW DATA ON LIMB DUPLICATION IN FROGS UNDER NATURAL CONDITIONS

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The phenomenon of duplication of the primordial hind limb of the pond frog, unique in the vastness of its scale, has been analyzed in a series of communications [2 5]. Material was collected from three unconnected natural ponds, situated in the foothills of the Zailiiskoe Ala-Tau, over a period of eight years beginning in 1947. A common feature of these ponds was an abundant supply of cold spring water. Abnormalities of development of the frogs caught in the different ponds were largely of one type: They were mainly limited to the region of the right hind limb, but extended in isolated cases to the region of the left limb also [5].

Our efforts to continue the study of this phenomenon have been unsuccessful in recent years on account of extensive improvement work in the area of these ponds. Examination of other ponds, especially those at a higher altitude, gave negative results, since the vast numbers of larvae and young frogs that were caught revealed no anomalies whatsoever.

Subsequently, therefore, we directed our main attention to the irrigated areas of the depression, situated at the same altitude as, or slightly below, the previous ponds. At the end of the summer and in the autumn of 1958, Z. A. Ivanova (laboratory assistant) found large numbers of anomalous late tadpoles and young frogs in two new ponds. These ponds were situated 6 km to the northeast of the main pond where our earlier investigations had been made [2]. Each of the two new ponds was supplied with cold spring water, which enters them abundantly from beneath the south bank, i.e. on the side of the nearest mountain range.

Four catches made in the second half of August and in September gave the following results: in the first, 122 specimens were caught, of which 16 (13.1%) were anomalous; in the second, 369 specimens were caught, of which 30 (8.1%) were anomalous; in the third catch, 34 of 344 specimens (9.8%) showed abnormalities; in the last, 50 specimens were caught, of which 6 (12%) were anomalous.

All the tadpoles with anomalies were greatly retarded in their general development. In this locality metamorphosis of the bulk of the larva of the pond frog is complete at the end of June to the beginning of July. At the catches made in the new ponds during August and September, a considerable part of the material consisted of tadpoles in different stages of development, in addition to young frogs. For instance, of the total number of 86 anomalous specimens which we collected, 57 were tadpoles and only 29 were young frogs of that year. Delay in general development was also shown by discrepancy in the growth of definitive organs and in the tempo of resorption of the larval organs: Growth of the hind limbs took place before resorption of the tail. We specially selected ten anomalous tadpoles (at the stage before appearance of obvious signs of resorption of the tail) and compared them with normally developing tadpoles with the same body and tail weight from the same ponds (see Table).

It can be seen from the Table that at a stage close to the full completion of metamorphosis, the weight of the hind limbs was approximately the same as the figure observed for the anomalous specimens, retarded in development, at an earlier stage. In the tadpoles possessing supernumerary limbs, the duration of proliferation of the main hindlimbs was thus increased, against a background of general delay in metamorphosis when the pond temperature was low (see Figure, a). We have shown earlier that an experimental increase in the duration of proliferation of the hind limbs has a strongly inhibitory action on metamorphosis [1].

Among the new material, seven main variations should be distinguished: The supernumerary limb was completely fused with the main limb as far as the paw – 34 specimens; the supernumerary limb was well differentiated and typically pigmented but was smaller than the main limb, for it was fused with the latter only in the region of the thigh – 18 specimens (solitary discoveries of this type have been described at various times [6]; on the right were three unequally differentiated hind limbs – 8 specimens (see Figure, 2); the supernumerary limb was not fused with the main limb but was attached in the region of the base of the right thigh, orientated in a lateral-cranial direction, well differentiated only in the distal portion and the skin depigmented

Comparative Data of Anomalous and Normally Developing Tadpoles

Conditions and stage of development	Weight (in mg)		
	of the whole larva	of the tail	of both hind limbs
Normal (stage III)	2 480	506	54
With a supplementary right limb weighing on the average 63 mg (stage III)	2 505	513	522
Normal (late stage V)	1 470	21	476

- 10 specimens; deformed supernumerary structures were fused with the proximal part of the thigh of the right hind limb - 11 specimens; the morphogenetic anomalies extended also to the region of the left hind limb - 2 specimens; the right hind limb was completely absent - 3 specimens.

A conspicuous feature was the predominance of cases of symmetrical duplication, as shown by the complete fusion of the ventral surfaces of the main and the supernumerary limbs; under these circumstances the two limbs may be indistinguishable from each other in size and morphological signs. In such cases the thigh and leg were joined together as mirror images, but in the region of the paw full union was not present and fusion was limited to the medial borders, sometimes with reduction of one or two of the medial digits. As a result, a conjoined fan was obtained, spread out in one plane, consisting of 10, 9, 8, 7 or 6 digits.

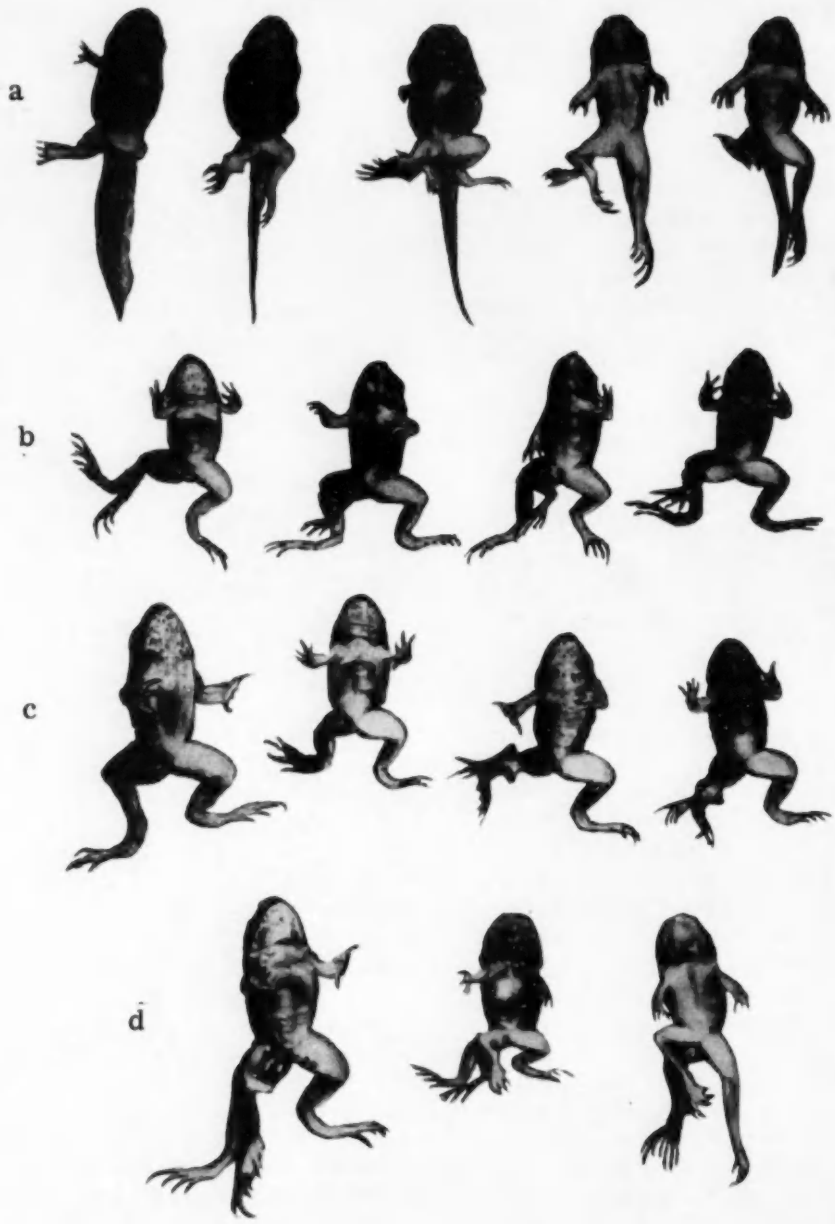
Such conjoined limbs had a common skin covering, highly pigmented over its whole extent according to the dorsal type (see Figure, c).

More than half the anomalous specimens with this mirror-image arrangement had a conjoined limb which was shortened by comparison with the single left limb. The shortening was due to the symmetrical transformation of the bones of the leg and the distal part of the thigh into spicular lateral processes in the two conjoined limbs. Altogether there were 22 specimens with spicular transformation of the middle part of the conjoined limb, or 25.6% of the total number of anomalies found (see Figure, c). Among the 304 anomalous specimens previously collected, 47 (15.4%) had this spicular deformity of the leg, predominantly affecting the right main limb. The supernumerary limb was often severely maldeveloped and very characteristically fused to the tissue of the main limb, so that one normal digit was incorporated as a sixth component of the paw of the main limb [4]. In the new material (see Figure, c), in 19 specimens (22.1% of the total number of anomalies) complete symmetry was observed, with an analogous spicular transformation of the leg of the supernumerary and main limbs (compared with 1.3% of the total number of 304 anomalies collected earlier). Only in three specimens was the spicular structure limited to the main limb, but in these cases too, the presence of a sixth digit in the paw of the main limb was not observed.

Another significant feature of the conjoined limbs in the new material was the normal orientation of the distal segments in the majority of anomalous specimens, whereas in 47 old specimens, possessing spicular structures, rotation of the paw through 180° was observed [4]. Rostans [7] observed in the green frog closely similar morphological deformities in the region of the leg and thigh and called them P anomalies. The main signs of these abnormalities were rotation of the paw in a cranial direction, transformation of the leg bone and part of the femur into a common, massive cone, severe shortening of the limb and, in several cases, the presence of a varying number of supernumerary digits in the paw. In such specimens a bilaterally symmetrical deformity of the right and left limbs was always observed. Comparison of our material with the anomalies shown in the numerous photographs accompanying the paper by Rostans [7] permits several signs of agreement to be recognized, but the morphological pictures are not completely identical.

In some cases, when the number of supernumerary limbs on the right side was two or three, we observed a gradation in their degree of development (see Figure, d); the least differentiated limbs were without pigment. An analogous phenomenon, although less well marked, may be seen in the photographs of some of the anomalous specimens (see Figures 27, 28 and 29 in the monograph by Rostans [7]), but a clear bilateral symmetry is observed here.

In 18 specimens the supernumerary limb was joined to the main limb only in the region of the thigh. In late tadpoles and newly developed young frogs such limbs were well differentiated, but were much smaller in size than the main limb (see Figure, b). At early stages of larval development the dimensions of the small supernumerary limbs were very little different from those of the main limbs. In the later period the supernumerary limb gradually falls behind in growth. This lag in growth is the more considerable the smaller the degree of fusion of the supernumerary limb with the main. For instance, in ten cases in which the supernumerary limb was in contact with the main limb only in the region of the pelvis or base of the thigh, only the distal parts of the supernumerary limbs were well formed, and in 11 cases shapeless fibro-osseous outgrowths developed, covered with depigmented skin. The trophic contact between the two limbs, restricted to the region of the thigh, ensured



Tadpoles and young frogs with supernumerary limbs. a) 5 anomalous tadpoles in various stages with massive hind limbs and delayed resorption of the tail; b) 4 young frogs with supernumerary limbs (fusion of the ventral surfaces restricted to the region of the thigh); c) complete fusion in the region of the thigh and leg (on the left – without deformity, on the right – with symmetrical deformity of the leg); d) the presence of a second supernumerary depigmented limb, situated beneath two other variously fused right limbs.

the harmonious development of the supernumerary limb, but with a lag in its growth that increased progressively with time (18 cases).

Close trophic contact between the two limbs, joined together in the region of the thigh and leg (34 cases), brought about growth of the supernumerary limb to roughly the same extent as growth of the main limb.

A common feature of the new material on anomalies and that which we collected and studied previously in the uniform character of the disturbance of morphogenesis in the region of the primordial right hind limb, which is found in variants from the local extinction of morphogenesis to the formation of several supernumerary limbs. The principle of duplication of the material of the anlage was revealed most clearly among the anomalous specimens of the new material. The level of morphological differentiation and the growth of supernumerary stnictures are directly dependent on the degree of anatomical fusion and trophic contact of the supernumerary and main limbs.

SUMMARY

A total of 86 new specimens of late tadpoles and young frogs (Rana ridibunda) with supernumerary right posterior extremities were caught in ponds. The growth and the morphological differentiation of the supernumery extremities depended directly on their anatomical connection of the supernumerary extremities with a spicular deformity of the shin was of a relatively frequent occurrence. Peculiar gradation was noted in cases of development of two and three supernumerary extremities.

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REGENERATION OF THE LIVER IN MICE IN EARLY PERIODS AFTER INJURY

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In a previously published work [1] we studied the mitotic activity in the liver after partial extirpation of that organ and its dependence on the time of the operation.

In the present work we investigated the changes in a number of morphological and cytochemical indices in the course of regeneration of the liver at different times of day after partial extirpation.

METHOD

From 60 to 70% of the liver (the central and left lateral lobes) was removed by the method of Higgins and Anderson from mice weighing 20-25 g. The weight of the part removed was 697-880 mg (3-4% of the body weight). The operations were carried out in February, in some of the mice in the morning (9 A.M. to 10 A.M.) and in others in the evening (6 P.M. to 7 P.M.). Altogether 90 mice were used, of which 65 were experimental and 25 controls.

The experimental and control animals were sacrificed 48, 54, 60, 66, and 72 hours after operation. The liver was weighed, the caudate lobe of the liver was fixed in Zenker's fluid with acetic acid, and the remaining part was fixed in 80° alcohol. The largest (D) and smallest (d) diameters of the liver cells were measured by means of an ocular micrometer in films stained by Gomori's method. Only mononuclear cells were measured, and only those cells were considered whose nuclei were in interkinesis. In each case we measured 100 liver cells. The diameter of the nuclei of the liver cells (d_n) was also determined. Measurements of the cell and nuclear diameters were made on the liver of 20 experimental and five control animals (in one animal for each period of fixation in mice undergoing operation in the morning and evening). Alkaline phosphatase was detected by Gomori's method and RNA was demonstrated by staining the films with methyl green pyronine.

RESULTS

On the second day after operation all the experimental mice lost from 2.5 to 5 g in weight. The absolute weight of the regenerating liver at this time was 780-1130 mg, or 4.2-5.7% of the body weight, and the weight of the liver in the control animals was 1190-1450 mg, or 5.5-6.1% of the body weight. These findings showed that the weight of the regenerating liver on the second to third day after operation reached 58-80% of the weight of the normal organ, irrespective of whether the operations were performed on the animals in the morning or evening.

Macroscopically, the lobes of the regenerating liver were considerably hypertrophied, although they had the normal dark red color.

The results of the measurements showed that the dimensions of the liver cells were increased on the second to third day after operation, and moreover that in the experimental mice undergoing operation in the morning, the smallest cell diameter d = 18.6μ and the largest D = 23.2 μ; in the control mice d = 17.7 μ and D = 20.7 μ; in the experimental mice undergoing operation in the evening, d = 20.5 μ and D = 24.1 μ, and in the controls d = 18.0 μ and D = 21.5 μ. An increase in the diameter of the nuclei was also observed. For instance, in the experimental mice undergoing operation in the morning, d_n = 8.7 μ, and in the controls d_n = 8.3 μ; in the experimental mice undergoing operation in the evening, d_n = 9.6 μ and in the controls d_n = 8.3 μ.

It can be seen from the figures in the table that hypertrophy of the nuclei and cells was more pronounced in the mice undergoing operation in the evening. The differences obtained were statistically significant. Hypertrophy of the liver cells in rats on the first day after operation was observed by several authors [2, 4, 6, 8]. In mice, however, observations in particular on the size of the liver cells at later periods after operation are not available. According to our findings, the hypertrophy of the liver cells was permanent in character.

Histological investigation showed that the regenerating liver was characterized by considerable polymorphism of the liver cells: side by side with very small cells, the diameter of which did not exceed 12.6 μ, were seen large cells, whose diameter was 36-43 μ; at the same

Results of Measurement of the Diameters of the Liver Cell and Nucleus After Partial Hepatectomy

Operation performed in morning, at 9–10 A.M.									Operation performed in evening, at 6–7 P.M.								
time of sacrifice after operation (hrs)	in hours	Experiment				Control			time of sacrifice after operation (hrs)	in hours	Experiment				Control		
	time of day at which fixation was carried out	smallest cell diameter, d (in μ)	largest cell diameter, D (in μ)	diameter of nucleus, d_n (in μ)	smallest cell diameter, d (in μ)	largest cell diameter, D (in μ)	diameter of nucleus, d_n (in μ)			time of day at which fixation was carried out	smallest cell diameter, d (in μ)	largest cell diameter, D (in μ)	diameter of nucleus, d_n (in μ)	smallest cell diameter, d (in μ)	largest cell diameter, D (in μ)	diameter of nucleus, d_n (in μ)	
48	9	18,4	22,9	7,4	17,6	21,2	8,4		48	21	21,2	23,8	9,3	18,3	20,5	8,0	
54	15	18,6	23,1	8,7	18,0	21,8	8,1		54	3	19,4	23,2	9,0	17,7	21,9	8,4	
60	21	18,6	22,6	8,7	18,3	20,5	8,0		60	9	19,3	24,1	8,4	17,6	21,2	8,4	
66	3	18,9	24,1	9,4	17,7	21,9	8,4		66	15	19,3	23,2	9,0	18,0	21,8	8,1	
72	9	18,7	23,1	9,0	16,8	20,7	8,3		72	21	21,2	26,1	10,4	18,3	21,8	8,3	
	M	18,6	23,2	8,7	17,7	20,7	8,3			M	20,5	24,1	9,6	18,0	21,5	8,3	

time cells could be seen with very large and with very small nuclei. These observations are also confirmed by data in the literature [3-10]. On the second to third day after operation, an enormous number of binuclear cells was found in the liver, and liver cells with three, or even four nuclei were also present. Cells in different phases of mitotic and amitotic division were observed (Fig. 1).

During investigation of the RNA and alkaline phosphatase content in the normal and regenerating liver, an uneven distribution of these substances in the cytoplasm of the cells of the parenchyma was observed. The liver cells of the marginal zone, cells situated near the blood vessels and at the periphery of the liver lobules, were richer in RNA and alkaline phosphatase. In the liver of the control animals daily changes were observed in the content and localization of RNA. In the morning (9 A.M.) and afternoon (3 P.M.) more RNA was present in the cytoplasm of the liver cells than in the evening and at night (9 P.M. and 3 A.M.).

Our findings were not in agreement with the biochemical investigations of Jardetzky et al. [8], who showed that the RNA content of the liver in normal mice is increased from 4 to 8 P.M. and thereafter gradually falls. In the hours of day, globules of RNA filled the whole cytoplasm of the liver cells, but in the evening and night hours they were situated mainly along the periphery of the cells, with a small amount left also around the nucleus. This phenomenon was evidently connected with diurnal changes in the liver glycogen content. In the course of the 24 hours the staining of the nucleoli with pyronine remained unchanged. In the normal liver cells alkaline phosphatase was present in very small amounts.

The time of operation (morning or evening) had no effect on the cytochemical changes observed in the regenerating liver, so that the findings which we describe were obtained in the two groups of animals.

No diurnal periodicity in the RNA content was observed in the regenerating liver of the mice. In the process of regeneration other regular features were observed in relation to the content of this compound, namely in the liver in which regenerative processes had not yet appeared or were ill-developed, the RNA content remained unchanged or was slightly reduced, and in these cases the activity of the enzyme was slightly increased; conversely, in the liver in which intensive proliferation of liver cells was taking place by mitotic and amitotic division, an increase in the RNA content and the enzyme activity was observed. At the same time a change took place in the character of the distribution of these compounds in the cytoplasm of the liver cells, whether undergoing mitotic division or newly formed; RNA and alkaline phosphatase were distributed here in the form of fine granules, filling the whole cell (Fig. 2).

In the regenerating liver at all times of fixation the cells of the bile ducts, sinuses and connective tissue contained a large amount of RNA and showed a strong en-

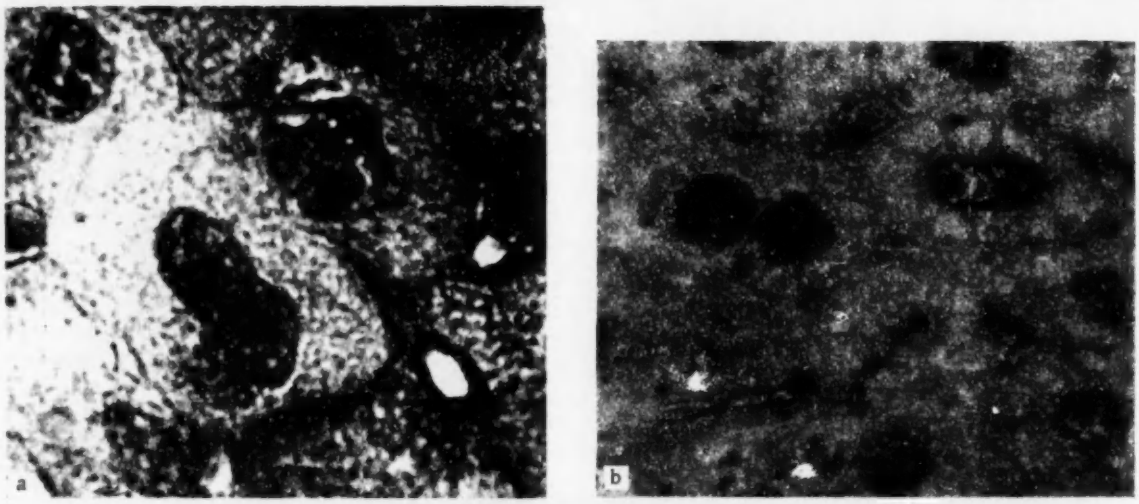

Fig. 1. Different stages of amitotic division in the regenerating liver of a mouse (on the second day). a) Constriction of the nucleus; b) dividing nucleus. Stained by Gomori's method for alkaline phosphatase. Magnification: ocular 6 ×, objective 90 ×.

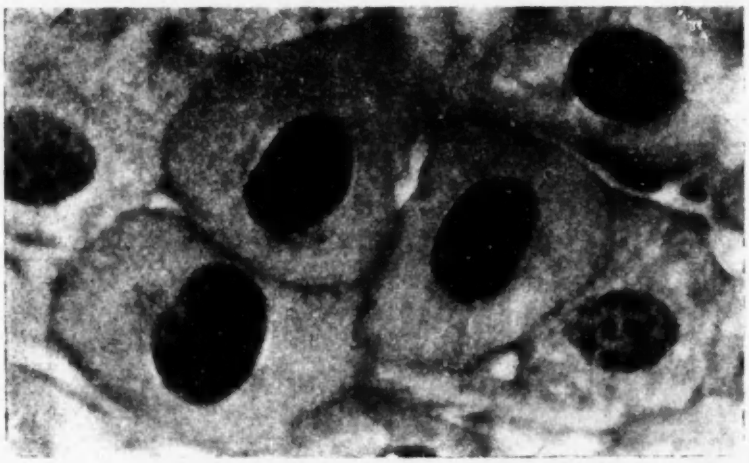

Fig. 2. A group of newly formed liver cells. Alkaline phosphatase in the form of tiny granules fills the whole cytoplasm of the liver cell. Stained by Gomori's method. Magnification: ocular 6 x, objective 90 x.

zymic reaction. The greatest increase in the RNA and alkaline phosphatase was observed 72 hours after operation (Fig. 3).

These findings were in agreement with the biochemical and histochemical investigations of Tsuboi and his coworkers [11-14].

In addition to newly formed cells, preserved liver cells with a normal content of RNA and alkaline phosphatase may be seen in the regenerating liver. The polymorphism of the liver cells during regeneration is thus increased still further by the considerable variations in the content of these compounds.

Side by side with the regenerative reaction in the liver of the mice on the second to third day after partial extirpation of the organ, destructive processes could be seen. These were primarily expressed in the presence of liver cells with a highly vacuolated cytoplasm. Cells were found with degenerative changes in their nuclei. In the process of destruction the picture of fragmentation and constriction of the nuclei could often be seen, and moreover this could not be confused with normal amitosis, for during the destructive changes the nuclear membrane acquired a ridged and uneven surface, the "amitosis" was not quite regular and the cytoplasm of such cells was highly vacuolated. The cells with degenerating nuclei also differed by their cytochemical characteristics from normal liver cells, the nuclei of which were undergoing amitotic division. Their cytoplasm contained little RNA and alkaline phosphatase.

In some cases it was difficult to differentiate the changes observed in the cells into regenerative or destructive processes. This concerned the distribution of

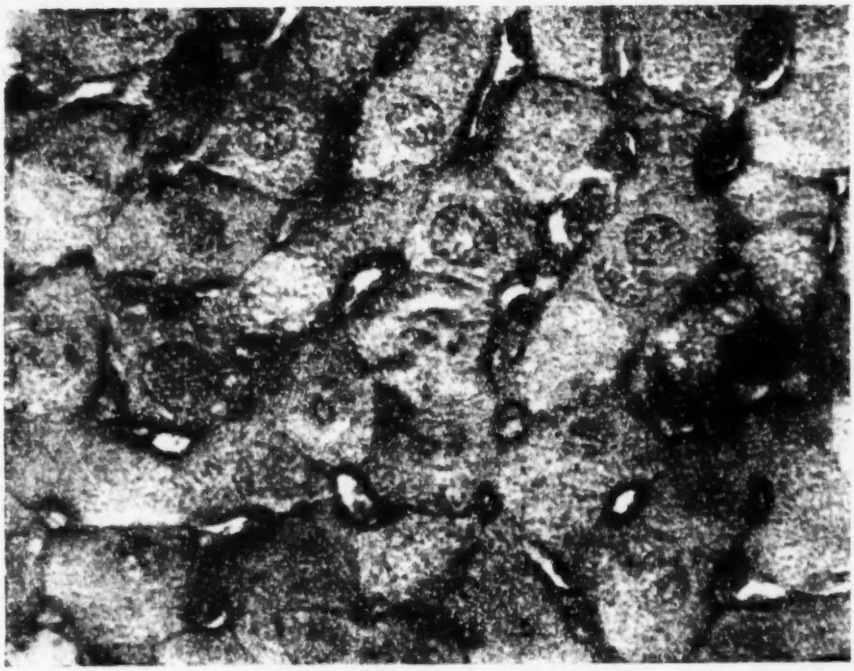

Fig. 3. Accumulation of alkaline phosphatase in the cells of the regenerating liver of a mouse (on the third day). Stained by Gomori's method. Magnification: ocular 5 x, objective 100 x.

the chromatin around the margins of the nuclei of the liver cells, and the vacuolation of the cytoplasm of certain liver cells, the nuclei of which were undergoing amitotic division, phenomena often observed in the regenerating liver.

In three cases large areas of necrosis of parenchymatous tissue were found in the regenerating liver, the liver cells being completely destroyed and staining badly with the ordinary histological stains, and considerable infiltration with blood cells being observed. Undamaged liver cells were preserved only around the blood vessels. No RNA nor alkaline phosphatase were found in the foci of necrosis.

SUMMARY

This work deals with the morphological and cytochemical changes in the regenerating liver of mice after removal of 65% of the liver tissue. Regenerative processes were found to occur shortly after the injury of the liver. Division of hepatic cells is both mitotic and amitotic. Hypertrophy of undividing hepatic cells continues for a period of 2-3 days. Cells adjacent to the blood vessels are more resistant to injury. The amount of RNA and alkaline phosphatase increases but their distribution in the cytoplasm of newly formed hepatic cells and in those undergoing mitosis is different. The time of the operation has no significant effect on the course of the regenerative process.

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THE POSSIBILITY OF INDUCTION OF MYELOID TISSUE IN IRRADIATED ANIMALS

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Myeloid tissue is distinguished by its high radiosensitivity. With a sufficiently large dose of whole-body irradiation, the injury to the bone marrow cells present in the body at the moment of irradiation is one of the most important links in the pathogenesis of radiation sickness. For this reason the attempts to transplant hemopoietic cells from healthy animals to irradiated recipients in order to replace the injured myeloid cells by repopulation of the bone marrow in the period preceding its regeneration are of such great interest [5]. One of the greatest difficulties in this procedure is caused by delayed secondary reactions, which are evidently due to immunological incompatibility of the foreign tissues [5].

The connective tissue of a normal animal is known to be capable of taking on a supplementary hemopoietic function, as is shown by the possible development of extramedullary hemopoiesis. The question arises whether the formation of myeloid tissue in an irradiated animal may be induced by a change in the direction of differentiation of those connective tissue cells which are relatively radioresistant and, in consequence of this, are not destroyed during irradiation sufficient to cause the death of the bone marrow cells.

Under experimental conditions the transitional epithelium of adult mammals possesses osteogenetic and myelogenetic activity [6, 7]. It has been shown [1-4] that substances with the histochemical properties of glycogen are secreted by transplantates of the mucous membrane of the urinary bladder and after ligation of the renal vessels, from the growing, undifferentiated layer of transitional epithelium into the surrounding tissue. This process is brought about as a result of buried areas of infiltration of this epithelium and of a specific secretory process, directed both through the surface of the layer and beneath it. The secretion of glycogen from the transitional epithelium is accompanied by induction of foci of ectopic bone formation and hemopoiesis in the adjacent connective tissue.

In the present communication we describe the findings concerning the inducing activity of transitional epithelium when transplanted into an irradiated recipient.

EXPERIMENTAL METHOD

The mucous membrane of the urinary bladder of guinea pigs, cut up finely with scissors, was homotransplanted into the anterior abdominal wall of 30 animals which had been irradiated 60-90 minutes beforehand with a dose of 250 r from a cobalt source EGO-2. After 8-21 days the transplantates were fixed by Helly's and Shabadash's methods and also with cooled acetone and were embedded in paraffin wax. Series of sections were stained with hematoxylin-eosin by the Dominici-Kodrovskii method, the periodic acid-Schiff method for alkaline phosphatase by Gomori's method and for RNA by Brachet's method.

EXPERIMENTAL RESULTS

Proliferation of the transplanted epithelium took place more intensively in the irradiated animals than in normal animals, and was accompanied by areas of buried infiltration. On the tenth day, in these areas, osteoblasts were differentiated from the connective tissue cells of the recipient and foci of ectopic bone developed. These had the typical appearance: The osteoblasts contained a large amount of RNA and alkaline phosphatase and the bone matrix showed the usual structure of integumentary anlagen. The inducing epithelium, as in transplantates in nonirradiated recipients [2-4], was rich in glycogen, which was secreted into the foci of induction. This process was accompanied by destruction of the cytoplasm of part of the epithelial cells invading the connective tissue; glycogen secretion could take place, however, without destruction of the cytoplasm of the epithelial cells, and it was directed both towards the underlying connective tissue and through the surface of the layer into the cavity of the epithelial cysts formed in the transplantates.

After the 17th day, foci of myeloid hemopoiesis were differentiated between the trabeculae of bone in the sites of induction, and on the 21st day actively proliferating hemopoietic tissue appeared here consisting of typical cells of the myeloid series with their charac-

teristic histochemical properties. The intensity of development of myeloid tissue in the irradiated animals was considerably in excess of that taking place by induction of hemopoiesis in transplantates in nonirradiated recipients. It was characteristic that, in contrast to the latter, in the irradiated animals myeloid tissue could also develop in the transplantates at points not in contact with osteogenic tissue, although always in direct proximity to transitional epithelium. On the 17th-21st day, transplantates were fixed from eight animals; in six transplantates induction of myeloid tissue was observed.

The histogenetic activity of transitional epithelium thus was apparent in irradiated animals also, in which it was shown in particular by induction of myeloid tissue. It is natural to consider that, immunologically speaking, the induced myeloid cells are not foreign to their own body. In this respect these findings concerning the possible induction of hemopoiesis in the connective tissue of irradiated animals by means of transitional epithelium may indicate one approach to the problem of the replacement of myeloid tissue injured by irradiation, by a method such as this in order to avoid delayed secondary reactions.

SUMMARY

Urinary blader mucosa was homotransplanted subcutaneously into guinea pigs irradiated with X-rays in a dose of 250 r, causing destruction of the bone marrow cells. Ectopic osteogenesis, with differentiation of foci of myeloid hemopoiesis, were observed in the irradiated animals under the effect of transplantation, this proving the preservation of the corresponding potentiality in the connective tissue of irradiated animals. The histogenetic activity of transitional epithelium is capable of inducing extramedullary myelopoiesis in irradiated animals.

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ON THE QUESTION OF THE PECULIARITES IN THE MORPHOLOGY OF THE VACCINAL TULAREMIA INFECTION IN ACUTE RADIATION SICKNESS

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In connection with the wide usage of ionizing radiation the question of the peculiarities in the morphology of the infectious process acquires actual importance. Indications are present in the literature that in the presence of radiation sickness an unusual course in the inflammatory process is observed, manifesting itself in the pronounced depression of the proliferative component of inflammation and predominance of certain hemorrhagic changes [1-3].

However, these investigations concerned themselves principally with the local inflammatory process. At the same time a study of the morphological course of inflammatory changes in generalized infections under conditions of radiation injury would present considerable interest, especially in the presence of infections characterized by definite specific morphological changes, particularly granule formation.

As a result of these considerations, we assigned ourselves the task of studying the peculiarities of the morphology of vaccinal tularemia infection in acute radiation sickness. As is well known [4-5], in vaccinal tularemia infection the same specificity of morphological changes is fundamentally retained as is seen with the administration of virulent tularemia bacteria.

METHOD

The experiments were carried out on 50 guinea pigs, whose average weights ran from 400 to 500 g. Part of the animals were subjected to a single total roentgen-radiation dose of 290 r DL 20 - 30/30 in the RUM-3 radiotherapy apparatus. Conditions of the radiation: 180 kv, 10 ma, 0.5 mm Cu and 1 mm Al filters, surface area 20 x 20 cm, focus distance 40 cm, dose energy 32 r/min. Twenty-four hours after the radiation, twenty pigs were injected intracutaneously, in the region of the righ hip, with 1 billion microbial cells of a 48-hour culture of B. tularense, prepared from dried tularemia vaccine obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology of the AMN SSSR. Two control experiments were set up: inoculation of an analogous dose of the vaccinal strain of bacteria without radiation (20 pigs) and radiation without inoculation (10 pigs); the irradiated control pigs were injected intracutaneously with physiologic saline. In addition, three normal pigs of the same weight, taken from the same group of animals as the experimental pigs, served as controls. Five pigs were sacrificed after 1, 3, 5, 7, 10, 14, 21, and 31 days post inoculation (two experimental, two inoculated controls, and one irradiated control). The investigated tissues were fixed in 10% formalin. The histologic preparations were stained with hematoxylin and eosin, and in isolated instances, by van Hieson's method. Blood was taken from each pig before it was sacrificed and determinations made of the number of leukocytes and the phagocytic index in relation to staphylococcus. In addition, beginning from the 14th day after inoculation, the titers of specific agglutinins were determined.

RESULTS

The dose of radiation used in the experiment caused death in 4 of the irradiated inoculated pigs and 2 of the irradiated control pigs. No deaths were observed among the inoculated control pigs.

Leukopenia developed in the irradiated pigs beginning from the fourth day after irradiation (3000 to 5000 per mm^3); the number of leukocytes decreased still further on the sixth day (to 2000 per mm^3), was maintained at this low level over the course of the eighth to eleventh day, rose on the fifteenth day to 5000 to 7000 and was restored to the normal magnitude by the 22nd day after radiation exposure. The degree of leukopenia was manifested approximately equally by the experimental animals (inoculated after irradiation) and the irradiated control animals which did not undergo inoculation. In the inoculated control animals on the 14th and 21st day after injection of the bacteria a minimally manifested increase in the number of leukocytes was noted.

In the experimental pigs and the irradiated control pigs on the eighth day after irradiation, the phagocytic index decreased to 7 - 15 against 18 - 23 in the inoculated control animals. Beginning from the eleventh day after radiation exposure and later it was not possible to evidence a distinct lowering of the phagocytic index in the experimental animals. As a rule, the titer of specific agglutinins did not change under the influence of radiation exposure. A decrease in the titer of antibodies in the irradiated inoculated pigs compared with the inoculated controls was seen only on the 21st day after irradiation.

We present the data of the morphological investigation.

Skin at the Site of Injection. A seropurulent inflammation is noted in the experimental animals in the dermis and subcutaneous cell layers 24 hours after inoculation. In the center of the focus of infection the walls of the small blood vessels are impregnated with plasma, this not being seen in the inoculated control animals. In the experimental animals, after 3 days, distinct necrotic changes are noted in the center of the nidus with a simultaneous productive reaction about the periphery in the form of reproduction of a considerable number of histiocytes; no essential differences are shown at this stage in comparison with the changes in the control pigs.

On the 5th day after inoculation, the necrotic changes in the zone of inflammation increase. The epidermis is necrotized. Massive foci of necrosis with nuclear disintegration are found in the dermis, as well as the subcutaneous tissue down to the muscle layer. At the periphery of the necrotic foci are observed numerous hemorrhages, edema of the blood vessel walls, and a productive-destructive panvasculitis. There is also noted a small productive reaction in the form of histiocyte reproduction. In the zone of the productive reaction around the blood vessels appear tularemia granulomas, consisting of epithelioid and plasma cells, fibroblasts, neutrophilic leukocytes and isolated lymphocytes. In contradistinction to the inoculated control animals, in the experimental animals at these time tintervals the alterative-necrotic changes predominate over the exudative-productive ones.

On the 7th and 10th day after inoculation, in the experimental animals, unlike the controls, there is still rather considerable necrosis with nuclear disintegration and hemorrhages along with an increase in the productive reaction and the formation of tularemia granulomas. By the 14th and 21st days after injection of the bacteria, in the skin and subcutaneous layers in between the separated elements of connective tissue, specific granulomas are still encountered, consisting of epithelioid cells, histiocytes, a small number of neutrophilic leukocytes, and multinucleated giant cells. The vaccinal tularemia skin changes are still not completed by the 31st day after inoculation. Here one can still encounter remnants of incompletely healed granulomas and even small leukocytic infiltrates. The impression is created that at a specific stage (from the 21st day after inoculation) the processes of healing at the site of injection of the vaccinal strain is inhibited somewhat in the experimental pigs as compared with the healing processes in the inoculated control animals.

Thus, in the skin of the vaccinated animals under the influence of irradiation, as opposed to the inoculated control pigs, beginning with the third day and up to the tenth day after inoculation more apparent necrotic-destructive changes are observed, involving all the elements of the skin including the walls of vessels. After the 10th day these differences level off and fall into the boundaries of individual variation in the reactions of animals. Furthermore, beginning with the 21st day the impression is created of a certain inhibition of the reparative processes in the experimental animals; in addition the morphological specificity of the vaccinal process is preserved in the experimental pigs (see figure).

In the irradiated control pigs 24 hours after the injection of physiological saline, edema and fine hemorrhages were seen in the dermis. In the following days a weakly expressed histiocytic reaction was observed, and after 14 days, a small scar.

Regional Lymph Nodes. No essential differences were noted in the experimental animals in comparison with the nonirradiated pigs after 24 hours. In both cases the appearance of a seropurulent lymphadenitis took place. In the experimental animals after 3 days a necrobiotic process developed in the lymphatic follicles; the nuclei of the lymphocytes underwent rupture and fusion. In the cortical layer under the capsule single miliary granulomas were encountered, consisting of reticular and epithelioid cells, neutrophilic leukocytes and single lymphocytes.

After 5 to 7 days, in the experimental pigs, along with the presence of miliary polymorphocellular granulomas in the cortical layer, were observed radiation changes manifested by a depression of lymphoid hematopoiesis and hemorrhages in the sinuses. In the inoculated control animals only polymorphocellular granuloma were noted.

After 10 to 14 days, numerous tularemia granulomas of different degrees of maturity were seen in the regional lymph nodes. In the experimental pigs were encountered coarse granulomas with widespread necrosis and nuclear disintegration in the center and a distinct specific productive reaction about the periphery in the form of growth of the epithelioid, reticular cells and fibroblasts. Other granulomas consisted basically of reticular, epithelioid cells and leukocytes. Dissimilar changes were noted in the lymphatic follicles: in some there was depression of lymphopoiesis; in others, on the contrary, there was development of reparative processes, characterized by the appearance in the follicles of small clear centers, made up of young lymphocytes.

After 21 to 31 days in the experimental pigs we also observed in one case wider necrotic changes in the tularemia granulomas in comparison with the control cases. The vaccinal changes were seen in a background of completely distinctive reparative-hyperplastic processes in the follicles.

Tularemia granuloma with giant cells in the skin of experimental guinea pigs 21 days after the injection of the vaccine. (Hematoxylin-eosin stain. Magnification: ocular. 7 x, objective 40 x).

Thus, in the regional lymph nodes of the experimental pigs the more frequently encountered alterative-necrotic changes can be noted in the tularemia granulomas in comparison with the inoculated control animals, with concomitant radiation changes and their sequellae.

The dynamics of radiation changes in the experimental pigs were not distinguishable morphologically from the changes in the irradiated control pigs.

The Spleen. In the experimental animals, as well as in the inoculated controls, in not a single case were specific tulareminal changes encountered in the spleen. Here the morphological differences were manifested in the state of the lymphoid apparatus. Just as in the regional lymph nodes, 3 days after inoculation a depression of lymphopoiesis in the follicles was noted in the experimental pigs. In them the clear propagating centers were not seen; in the central parts of the follicles there was the appearance of karyolysis and karyorrhexis in the lymphocytes. Subsequently, up to the 14th day progression of these processes was observed. A substantial hemosiderosis appears. Beginning with the 21st day, along with the destruction of the lymphoid tissue in numerous follicles and partial replacement of the latter with connective tissue, the signs of restoration of lymphopoiesis are noted in the preserved follicles. This is expressed in the formation of light centers in the follicles, consisting of young lymphocytes, and in the restoration of normal follicle dimensions.

Comparing the appearance revealed with the changes in the irradiated control pigs, it is possible to conclude that all the described morphological changes in the spleen of the experimental animals were related to irradiation.

In the inoculated control animals, as opposed to the experimental pigs, there was observed a typical nonspecific infection-type of hyperplasia of the splenic pulp.

Liver and Kidneys. Under the influence of ionizing radiation there early develops a granular dystrophy of the parenchymatous elements in the liver and kidneys.

The data obtained allows arriving at the conclusion that massive roentgen radiation of guinea pigs, causing acute radiation sickness with a mortality outcome of 20 to 30% of all cases, produced previous to injection into the skin of massive doses of the vaccinal strain of tularemia bacteria, does not change the morphological specificity of the vaccinal process. However, in addition, all this causes certain morphological peculiarities, demonstrated in the fortification of the alterative component of inflammation in the skin and regional lymph nodes. In the cases described by us, these peculiarities

were not as markedly manifested as those observed by other authors [1-3] with the action of ionizing radiation on a nonspecific nidus of inflammation. Despite the indicated morphological differences, the clinical course of the vaccinal process in both the experimental and the control animals did not essentially differ.

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METHODS

A MODIFICATION OF THE METHOD USING PRECIPITATION IN AGAR TO COMPARE TWO ANTIGEN-ANTISERUM SYSTEMS

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Original article submitted July 13, 1959

The method using precipitation in agar became particularly effective after Ouchterlony modified it to compare complex antigens with each other [4]. The fact that this modification permits one to establish the immunological affinity of the components of complex systems, as well as to determine the number of antigens in a system, makes it particularly important.

Different variations of Ouchterlony's method are in use, most of which are essentially an elementary system consisting of two comparable antigens diffusing towards a serum or, conversely, to antisera diffusing towards one antigen [3]. Whith this arrangement, an antigen found only in one system forms a "spur" with the homologous serum, which does not run together with the precipitation spectrum of the heterologous system.

In many cases, however, depending chiefly on the strength of the antigen and serum as well as on the complexity of the spectrum of the preparations being compared, it is difficult to determine the true "spur" and to distinguish it from a false one caused by a difference in the concentrations of the antigens being compared. This difficulty is aggravated by the fact that the "spur" forms in the zone farthest from the diffusion centers, so that these most important parts of the spectrum are often not clearly apparent.

We often encountered these difficulties in work with tissue antigens and therefore developed a simple modification of Ouchterlony's method in order to more clearly demonstrate specific antigens. With the use of this modification, the precipitation zones are situated so that the whole length of the line of the antigen specific to one system only differs in direction from the spectrum of the antigens common to the preparations being compared and crosses this spectrum in the optimal reaction zone. In addition, this modification permits the comparison of two complete antigen-antiserum systems at once.

Two systems of antigen and its corresponding serum (AC-ac and BC-bc, where A, B, C are the antigenically unrelated components and a, b, c are their corresponding antibodies) are arranged crosswise at opposite corners of a square (Fig. 1, I).

The presence of other, unrelated antigens, antibodies or the precipitates formed by them in the agar does not affect the reaction of a specific antigen-antibody pair. Therefore, in the interest of clarity, the reaction of each antigen-antibody pair can be considered separately as if it had occurred in an isolated system.

Figure 1, II shows the reaction between antigen A and its antibody a. The precipitate Aa forms where the diffusion zones of A and a (depressions 1 and 3) meet; because depressions 2 and 4 do not contain related components, the preparations in them do not affect the position of Aa. With optimal A and a correlations, the precipitate line passes through the center, and its ends rest on the depressions of the heterologous system. If, however, there is a large surplus of one of the components, the line passes below or above depressions 2 and 4, and the ends of the line Aa become somewhat distorted.

The phenomenon is demonstrated in Fig. 1, III. The antibody, when there is a greater amount than the antigen A, diffuses and enters the fluid-filled depressions 2 and 4, mixing into them. Owing to this, part a, diffusing through the depressions, overlaps the common diffusion front in the agar, and the portions of zone A adjoining depressions 2 and 4 bend under symmetrically, as shown in Fig. 1, III.

The above is also true of system B-b, the precipitate of which is located between depressions 2 and 4, at right angles to Aa (Fig. 1, IV). In the presence of an antigen common to the preparations being compared (C) and antibodies to it (c), the line of precipitation runs in a different direction (Fig. 1, V). This is because C, diffusing simultaneously from two depressions (1 and 2) forms a common antigen front, moving from left to right to meet the common front of the antibodies diffusing from the serum depressions 3 and 4. The precipitate which forms at the meeting place of the fronts Cc, therefore, is located in the zone between the antigen and serum depressions.

Therefore, if two systems which have a common antigen, as well as one specific to each system, are compared, the lines of the precipitates for the specific antigens form a cross, while the precipitate line formed for the common

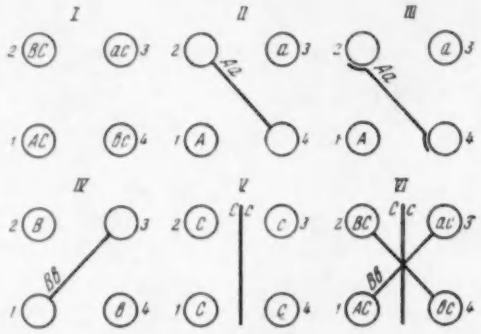

Fig. 1. Schematic representation of the modified precipitation-in-agar reaction (see text for explanation).

Fig. 2. Comparison of two systems with common antigens and antigens specific to each system. N) Antigen from mouse liver; H) antigen from mouse hepatoma; AN) antiliver serum; AH) antihepatoma serum.

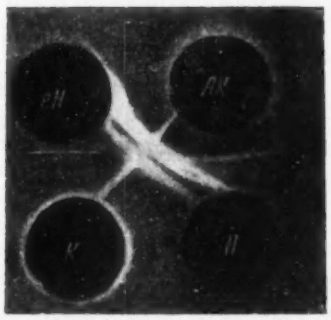

Fig. 3. Nonidentity reaction of specific hepatoma antigen with kidney antigens. H) Antigen from mouse hepatoma; K) antigen from mouse kidney; eH) antibodies to specific hepatoma antigen; AK) antikidney serum.

antigen is vertical (Fig. 1, VI). It is obvious that the same figure is also formed in more complex systems.

The proposed experimental arrangement has certain advantages over the usual arrangement. In the first place, it facilitates the demonstration of qualitatively different antigens, because the whole length of the lines formed by such antigens differ in direction from the spectrum of common antigens, which these lines cross in the optimal reaction zone, i.e. at the shortest distance from the centers of diffusion. In the second place, the proposed arrangement permits one to compare two complete antigen-antiserum systems and to obtain of full serological characterization of them simultaneously. Moreover, the lines of a specific precipitate form only if analogous antigen and the antibodies to it are not present in the heterologous system, which creates more rigid and objective criteria of the specificity of the given component.

We have used this modification extensively in the study of organospecific and specific tumor antigens [1,2].

Figures 2 and 3 show examples of the modified precipitation-in-agar reaction, using different antigens and corresponding antisera.

SUMMARY

Antigens and antisera are placed in depressions inside the angles of a square. Each antigen is located diagonally opposite the homologous serum. The antigens specific for each system give precipitation lines crossing at the center of the square at right angles to each other, their ends resting against the depressions of the heterologous system. The antigens common for the systems under comparison produce lines passing between the reservoirs of both antigens and sera. This method facilitates the detection of qualitatively different antigens in the preparations under comparison and permits the analysis of two complete antigen-antiserum systems.

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*Original Russian pagination. See English translation.

A PURIFIED ENZYME PREPARATION OBTAINED FROM SOVIET PANCREATIN AND ITS USE FOR TISSUE "TRYPSINIZATION"

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(Presented by Active Member of the AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' éksperimental'noi biologii i meditsiny Vol. 49
No. 3, pp. 120-121, March, 1960
Original article submitted February 4, 1960

The preparation trypsin-Difco [4, 5], which is not highly purified trypsin, but a mixture of the latter, chymotrypsin and certain other pancreatic enzymes, is usually used in the preparation of cell suspensions for tissue cultures to be used for tissue "trypsinization". Therefore, the terms "trypsin" and "trypsinization" are in this case conditional. Recently, an extract of nonpurified pancreatin [1], the dosage of which was not determined according to its activity, has been used for tissue "trypsinization", but, as our works have demonstrated, the activity and the protein nitrogen content of pancreatin extracts vary considerably from series to series. For example, we found that the activity of 10% aqueous extracts of pancreatin, determined according to the Chow-Peticolas method [3], ranged from 2,000 to 15,000 Chow/ml (Chow units per 1 ml).

We took on the task of developing a simple, quick and practicable method of obtaining purified preparations containing trypsin and other pancreatic enzymes, the activity of which would only vary slightly.

We proposed a scheme for obtaining a purified enzyme preparation by processing the pancreatin extract twice with activated charcoal (brand A, All-Union State Standard 4453-48).

We used the Chow-Peticolas method [3], which is based on turbidimetric determination of the degree of casein proteolysis, to demonstrate proteinase activity. As the Chow unit, we took the amount of the enzyme which could in 15 minutes (at 37-38°) half of the equal volume of a 0.25% casein solution into a state of unprecipitability by 5% trichloracetic acid.

The casein concentration was determined according to the absorption of light by its suspensions. The protein nitrogen was determined by Kjeldahl's method.

The original pancreatin preparation (series No. 35) was obtained from A. I. Mikoyan's Myasokombinat [Meat combine, literally].

Method of Preparing Preparation. One hundred and twenty-five g dry pancreatin was suspended in 1 liter distilled water; the suspension was shaken thoroughly for an hour, and the sediment was separated by centrifugation. The supernatant liquor containing 100-150 mg % protein nitrogen and 2000-2500 Chow/ml.

We added 30 g activated charcoal (first washed with distilled water) to 1 liter of the supernatant liquor, shook the mixture for 10 minutes and separated the sediment by centrifugation. The operation with the charcoal was then repeated. After the second separation of the charcoal, the solution was clarified by filtering through a 2-3 cm layer of paper pulp on a Buchner's funnel. The clarified solution contained 30-50 mg% protein nitrogen and 750-1800 Chow/ml; the pH of the solution was 5.0-6.5.

The solution was subjected to sterilizing filtration through F-5 candles, after which it was poured into ampules and stored at +4°. Neither the sterilizing process nor 6-month storage caused any significant decrease in the proteinase activity.

The activity of this enzyme preparation is essentially the same as that of crystalline trypsin and chymotrypsin. Each purification cycle requires only one working day and a few readily available reagents. The preparation can be stored for 6 months (observation period). Before use, the enzyme preparation is poured out of its ampoule and diluted with a sterile Henke's saline solution (pH = 7.2-7.6) to the necessary activity (50-100 Chow/ml). This working solution will remain suitable for use for 2-3 weeks at +4°.

For tissue "trypsinization", small pieces of organs taken from animals under sterile conditions are rinsed free of blood in a Henke's saline solution to which 50-100 units of penicillin per 1 ml of fluid has been added, cut into pieces 1 mm^3 in size with a razor and then again rinsed in the saline solution. The pieces are then transferred to a flask; the working solution of "trypsin" is poured over them in a ratio of 5:1, and the combination is shaken up in a shuttle-apparatus for 15 minutes at 36-37°. After the pieces have settled to the bottom of the flask, the liquid portion containing blood and scraps of tissue is removed and replaced with the same amount of fresh "trypsin" solution; the flask is then shaken again for 30-60 minutes. The length of the "trypsinization" process depends on the age and type of the animals, as well as on the structure of the organ. One and a half to two hours are required to "trypsinize" the tissues of adult animals (the kidneys of an adult rabbit, for example).

Human embryonal tissue breaks down into cells in 30-60 minutes. After the "trypsinization", the contents of the flask are centrifuged for 5 minutes (1000 rpm) at room temperature. The supernatant liquor is discarded, and the sediment is resuspended in a fluid nutrient mixture containing, besides Henke's saline solution (50%), human or horse serum (25%) and rabbit extract of the spleen and bone marrow in a dilution of 1:50 (25%). The nutrient mixture, in which cells are suspended individually and in complexes of 10-20, is poured in amounts of 1.5 ml into test tubes. Test tubes of neutral glass are preferable for this purpose. Mica flakes, 5 x 1.5 cm in size, are bent into a U-shape so that they conform almost exactly to the test tube walls and inserted into the test tubes before the sterilization of the latter to aid in the ensuing detailed morphological study of the "trypsinized" tissue cultures.

During the first 24 hours, cultivation at 36-37° is carried out with the test tubes in a semi-horizontal position. During this period, the cells settle on and adhere to the test tube walls. On the second day of cultivation, the test tubes are put into a drum [see 2] rotating at a rate of 16 rev/hour. When prolonged cultivation of the tissues is required, the "trypsin" solution is also used to produce transplants. For this purpose, the solution is heated to 36°, poured into the test tubes with the cells, from which the nutrient mixture has been first removed, and left for 5 minutes at 36° (can also be left at room temperature if the period of incubation with the "trypsin" solution is lengthened correspondingly). The test tubes are shaken up so that the cells are dislodged from the test tube walls, in sheets or cell complexes in the case of epithelial tissue growth, and usually in single cells when there is fibroblastic growth. The suspension is poured off into centrifuge test tubes and centrifuged under the conditions described above. The supernatant liquor is discarded, and the sediment is transferred to the nutrient mixture, the material of each test tube being divided into 2 or 3 parts depending on the previous growth of the cells.

The use of an enzyme preparation, the dosage of which can be exactly determined according to its proteolytic activity, makes it possible to obtain reproducible results.

Our "trypsin" enzyme preparation is as strong as trypsin-Difco in ability to "disperse" cells and can be effectively used in the practice of tissue cultivation.

SUMMARY

The authors obtained an enzyme preparation - a mixture of tryspin, chymotrypspin and other pancreatic enzymes from the extract of Soviet pancreatin by subjecting it to double purification through activated charcoal. The activity of the resulting preparation approaches that of the crystalline tryspin and chymotryspin (750-1800 Chow Units/ml and 30-50 mg% protein nitrogen). The enzyme preparation may be stored in solution in ampules for a period of 6 months at a temperature of +4°C without any noticeable loss of its effectiveness. The preparation successfully replaces trypsin-Difco in tissue cultivation. Its dosage was determined not by the weight of the dry pancreatin, but by the activity expressed by the proteolytic "Chow" Units, which enabled the authors to obtain reproducible results.

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ERRATA

Vol. 49, No. 1, August, 1960

Page	Column	Line	Reads	Should read
29	left	24 from top	900-100 mm	90-100 mm
29	left	10 from bottom	(−20 °)	(20°)
35	left	13 from bottom	deafferentation and deafferentation	deafferentation and deefferentation
36	left	14 from top	deafferented limb	deefferented limb
36	left	17 and 18 from top	deafferented limb	deefferented limb
36	right	17 from top	deafferented left hind limb	deefferented left hind limb
36	Table 2, column 2 group 2		Toxin in muscles of deafferented limb	Toxin in muscles of deefferented limb
37	Fig. 2 caption	2	a deafferented one (2),	a deefferented one (2),
38	left	18 from top	a deafferented limb	a deefferented limb
38	left	21 from top	of the deafferented limb	of the deefferented limb
88	left	last 4	A typical muscular.....	A typical muscular organ was formed as a result of restorative process. Necrosis of minced muscular fibers was intensified in the presence of purulent inflammation; the progressive development and the resolution of necrotic particles became sharply retarded and the productive development of connective tissue, resulting in the formation of connective tissue reproduction at the site of the transplant, was stimulated.
	right	first 5	.....was stimulated	

The title of the article beginning on page 101 should read

TECHNIQUE OF EMBEDDING DRY ANATOMICAL AND BIOLOGICAL SPECIMENS IN ORGANIC GLASS (METHYL METHACRYLATE)

SIGNIFICANCE OF ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN SOVIET PERIODICALS

FIAN	Phys. Inst. Acad. Sci. USSR.
GDI	Water Power Inst.
GITI	State Sci.-Tech. Press
GITTL	State Tech. and Theor. Lit. Press
GONTI	State United Sci.-Tech. Press
Gosenergoizdat	State Power Press
Goskhimizdat	State Chem. Press
GOST	All-Union State Standard
GTTI	State Tech. and Theor. Lit. Press
IL	Foreign Lit. Press
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd. AN SSSR	Acad. Sci. USSR Press
Izd. MGU	Moscow State Univ. Press
LEIIZhT	Leningrad Power Inst. of Railroad Engineering
LET	Leningrad Elec. Engr. School
LETI	Leningrad Electrotechnical Inst.
LETIIZhT	Leningrad Electrical Engineering Research Inst. of Railroad Engr.
Mashgiz	State Sci.-Tech. Press for Machine Construction Lit.
MEP	Ministry of Electrical Industry
MES	Ministry of Electrical Power Plants
MESEP	Ministry of Electrical Power Plants and the Electrical Industry
MGU	Moscow State Univ.
MKhTI	Moscow Inst. Chem. Tech.
MOPI	Moscow Regional Pedagogical Inst.
MSP	Ministry of Industrial Construction
NII ZVUKSZAPIOI	Scientific Research Inst. of Sound Recording
NIKFI	Sci. Inst. of Modern Motion Picture Photography
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
OTN	Div. Tech. Sci.
Stroiizdat	Construction Press
TOE	Association of Power Engineers
TsKTI	Central Research Inst. for Boilers and Turbines
TsNIEL	Central Scientific Research Elec. Engr. Lab.
TsNIEL-MES	Central Scientific Research Elec. Engr. Lab.–Ministry of Electric Power Plants
TsVTI	Central Office of Economic Information
UF	Ural Branch
VIESKh	All-Union Inst. of Rural Elec. Power Stations
VNIIM	All-Union Scientific Research Inst. of Meteorology
VNIIZhDT	All-Union Scientific Research Inst. of Railroad Engineering
VTI	All-Union Thermotech. Inst.
VZEI	All-Union Power Correspondence Inst.

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. – Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Ekspti. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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SOVIET research in PHARMACEUTICAL CHEMISTRY | 1956

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